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The role of pedunculopontine nucleus in choice behavior under risk

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Abstract

The dopaminergic projections to the basal ganglia have long been implicated in reward guided behavior and decision making, yet little is known about the role of the posterior pedunculopontine nucleus (pPPN), a major source of excitatory input to the mesolimbic dopamine system. Here we studied the contributions of pPPN to decision making under risk, using excitotoxic lesions and reversible inactivation in rats. Rats could choose between two options: a small but certain reward on one lever, or a large but uncertain reward on the other lever. The overall payoff associated with each choice is the same, but the reward variance (risk) associated with the risky choice is much higher. In Experiment 1, we showed that excitotoxic lesions of the pPPN before training did not affect acquisition of lever pressing. But whereas the controls strongly preferred the safe choice, the lesioned rats did not. In Experiment 2, we found that muscimol inactivation of the pPPN also reversibly altered choice behavior. These results show that permanent lesions or reversible inactivation of the pPPN both abolish risk aversion in decision making.

Keywords

pedunculopontine nucleus; choice; operant; reward; risk; basal ganglia; decision making; dopamine

Introduction

We often face the choice between small and certain rewards and large and uncertain rewards, e.g. 100% chance of getting 100 dollars or 10% chance of winning 1000 dollars. The latter option is said to be more risky. Risk can be formally defined as the variance in a desired outcome (Leblond et al., 2011). As such it is known to influence decision making, not only in humans but in all animal species studied (Kacelnik and Bateson, 1997; Preuschoff et al., 2006; Glimcher, 2008; Platt and Huettel, 2008; Simon et al., 2011; St Onge et al., 2011). This is hardly surprising, since risk is a universal feature of the natural environment. For most organisms, desired commodities do not come in constant, uniform packages. As a result of uncertainty in the environment and variations in the consequences of behavior, risk is associated with most choices.

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The neural substrates of risk-based decision making, however, remain poorly understood. Although studies have implicated the mesolimbic dopamine system and associated regions in risk-based decision making (Cardinal and Howes, 2005; St Onge et al., 2012), little is known about the specific contributions of the relevant brain regions. To elucidate the neural mechanisms underlying risk-related decision making, this study examined the role of the pedunculopontine nucleus, a major source of excitatory input to the mesolimbic dopamine system. The pedunculopontine nucleus receives extensive inputs from the basal ganglia and sends projections to midbrain dopamine neurons. The posterior pedunculopontine nucleus (pPPN), in particular, projects to DA neurons in the ventral tegmental area (VTA) (Winn, 2008; Wilson et al., 2009; MacLaren et al., 2013).

We examined the effects of excitotoxic pPPN lesions on choice behavior under risk, using a discrete-trial operant choice task to measure risk preference (Leblond et al., 2011). In this task, two choices yield the same overall payoff. The safe choice, however, is always followed by a small reward, whereas the risky choice is associated with the same mean reward rate but higher variance.

Previous human imaging work has implicated the mesolimbic dopamine system and its target brain regions in immediate gratification or exploitation (McClure et al., 2004; Cohen et al., 2007). As the safe option is associated with immediate gratification, given the pPPN projections to the dopamine neurons, we predict that lesion or inactivation of the pPPN will reduce risk aversion.

Materials and Methods

Subjects

All procedures are in accordance with the Guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedure and approved by the Duke University Animal Care and Use Committee (protocol A162-11-03). For all experiments, Long-Evans rats (~3-6 months of age, Charles River Laboratories) were used. They were housed in pairs, under a 12/12 light/dark cycle.

Experiment 1: Posterior pedunculopontine (PPT) lesion

Surgical and training procedures are similar to those described previously (Yu et al., 2010). Rats were anesthetized with isoflurane (1-2%) and placed on a stereotaxic frame. The following coordinates (in mm) were used: anteroposterior -8.3 mm, mediolateral +/-2, dorsoventral - 6.8 mm, relative to bregma. Bilateral lesions (n = 10) were created by injecting N-methyl-D-aspartic acid (NMDA, 0.1 M in phosphate buffered saline, PBS) at a rate of 0.1 µl/min over 2 minutes using 0.5 µl Hamilton syringes. The needles were then kept in place for 5 minutes to allow for drug diffusion. Sham lesions (n = 9) were created using the same procedure, except only PBS solution was injected.

All rats were given two weeks to recover from surgery before starting the experiments. One week before the start of the behavioral experiments, they were food deprived. Their body weight was monitored daily and maintained at ~85-90% of free feeding weight (~10-12 g of

home chow per animal, given ~2 hours after each behavioral session). Water was always available in the home cages.

Instrumental training

Experiments took place in Medical Associates operant chambers, as described previously (Yu et al., 2010). Each chamber contains two retractable response levers, located on either side of a food magazine, where pellets can be dispensed. Initial lever press training consisted of 3 continuous reinforcement (CRF) sessions, in which each lever press resulted in the delivery of a pellet (45 mg, Bio-Serv) into the food cup. The rats were then trained under a fixed ratio 5 (FR5, one pellet every 5 presses) schedule for 3 additional days. The sessions ended after 1 hour or the delivery of 30 pellets.

Discrete trial choice task

Following initial instrumental training, rats were trained on the discrete trial operant choice task. They could choose between a safe lever and a risky lever. Pressing the safe lever is followed by a small, constant reward (1 pellet), while pressing the risky lever is followed by a large, uncertain reward (8 pellets with probability of 0.125). Based on previous work from our lab that had characterized the effects of different risk levels on choice behavior (Leblond et al., 2011), we deliberately chose a very high level of risk, which can produce reliable risk aversion. The high level of risk aversion in normal rats under these conditions would then allow us to test the hypothesis that pPPN lesions reduce risk aversion.

Each session lasted 2 hours. At the beginning of each session, the light was turned on and two levers were inserted. Once levers were presented, they remained available until pressed. As soon as the rat made a choice by pressing one of the levers, both levers were retracted, and the light was turned off. The inter-trial-interval (ITI) lasted 40 seconds, which was always long enough for the rats to collect and consume the pellets. Because the rats were initially trained on the left lever, to eliminate any lever preference the right lever was always used as the safe lever and the left lever used as the risky lever. All rats were trained for 10 daily sessions, until their performance across the two levers remained stable for at least 3 sessions.

Histology

After the completion of behavioral testing, all rats were anesthetized with isoflurane and perfused transcardially with 10% formalin solution. 120 μ m coronal sections of their brains were cut with a Vibratome 1000 Plus. The brain sections were then mounted on slides and stained with thionin, and examined under a microscope.

Experiment 2: Reversible inactivation of pPPN

Cannulae implantation

Twelve rats were first trained on lever pressing and the decision task as described above. They were then implanted with guide cannulae in the pPPN. The skull was exposed, and bilateral holes were drilled using the following coordinates: anteroposterior -8.3 mm, mediolateral $\pm$ 2 mm, dorsoventral -6.8 mm, relative to bregma (Paxinos and Watson,

2007). Stainless steel guide cannulae (24-gauge, 0.29 i.d., 7 mm length) were lowered 6 mm below the brain surface (~1 mm above the target area). The cannulae were secured using stainless steel screws and dental acrylic cement. Custom-made wire stylets were inserted into the guide cannulae to prevent clogging. Rats were given 5-8 days to recover, after which they were again food-deprived and re-trained on the discrete trial choice task. The procedure is the same as in Experiment 1, except that each choice behavior session lasted 60 min instead of 120 min. The session length was reduced to ensure that muscimol was effective throughout the entire session. The assignment of the safe lever and risky lever was identical to that prior to surgery.

Choice reversal

There could in principle be natural biases in the choice of the lever. To address this concern, we also tested the ability of the animals to reverse their choice behavior on the two levers. The right lever, which was used as the safe lever, was changed to the risky lever, and vice versa for the left lever.

Intracerebral microinfusions

Muscimol (5-aminomethyl-3-hydroxyisoxazole; Tocris), a GABA_A receptor agonist, was used to reversibly inactivate the pPPT. The drug was dissolved in phosphate buffered saline (PBS). The desired dose was prepared from the stock solution on the day of injections. Rats were anesthetized using isoflurane (as before) and attached to the nose piece of the stereotaxic frame. Stylets were removed. Two injection needles (1mm below the tip of the guide cannulae) attached to polyethylene tubes delivered the injections. The tubes were attached to two 1µl Hamilton syringes which were fixed to a micro-infusion pump (Harvard Apparatus, Holliston, MA, USA), delivering 0.6µl of 1.6 mM muscimol over 6 minutes. The injection needles were kept in place for an additional 60-90 seconds to allow for diffusion, then removed and replaced by the stylets. Rats were given 30 minutes to recover, then tested on the decision task as before. Control injections were done in the same way, except the same volume of PBS solution was injected. All rats served as their own controls.

Data analysis

Data analysis and statistical tests were performed using Matlab and Graphpad Prism.

Results

The extent of excitotoxic lesions and cannula placement

After the completion of experiments, all rats were sacrificed and their brains examined for lesion and cannula placement. Fig. 1A shows representative coronal sections from sham-lesioned controls and pPPN-lesioned rats. We used the criterion established by a previous study (Alderson et al., 2008) to determine the extent of pPPN lesions. In the lesion group, small and bilateral lesions, characterized by neuronal loss and gliosis, were observed in all but three rats, who had either unilateral or more anterior lesions. These 3 rats were excluded from the analysis (final group sizes: n = 9 for sham controls, and n = 7 for pPPN lesions).

Lever press acquisition

Using the rate of lever pressing as a measure, we compared the control group with the lesion group in initial operant conditioning (Fig. 1C). We did not observe any difference in the rate of lever pressing. A 2-way ANOVA with Time and Lesion as factors revealed a significant main effect of Time ($F_{5, 70} = 48$, $p < 0.0001$), but no main effect of Lesion ($F_{1, 70} = 0.08$, $p < 0.05$), and no interaction between these factors ($F_{5, 70} = 2.0$, $p < 0.05$). Nor did we observe any differences in the rate of head entries into the food magazine (Fig. 1D). There was a significant main effect of Time ($F_{5, 70} = 21.2$, $p < 0.0001$), but no main effect of Lesion ($F_{1, 70} = 1.1$, $p > 0.05$) or any interaction between them ($F_{5, 70} = 0.53$, $p > 0.05$). Thus, rats from both groups could learn to press a lever for food rewards, increasing their rate of pressing over time. The lesioned rats did not show any gross deficits in sensory processing and motor control.

Choice behavior under risk

During each choice behavior session, the lesioned rats showed dramatically reduced choice of the safe lever and increased choice of the risky lever (Fig. 2). This observation was confirmed by statistical analysis. The number of lever presses on each lever was averaged across the last 3 days of testing (Fig. 2B). A 2-way ANOVA with Lesion and Risk as factors showed no main effects of Lesion ($F_{1, 14} = 2.55$, $p > 0.05$) or Risk ($F_{1, 14} = 2.14$, $p > 0.05$), but a significant interaction between these factors ($F_{1, 14} = 7.93$, $p < 0.01$). Post hoc tests revealed a significant effect of lesion on each of the two choices. The control group made significantly more safe choices than the lesion group ($p < 0.01$), whereas the lesion group did not ($p > 0.05$). On the other hand, the overall number of pellets earned did not differ between control rats and lesioned rats (unpaired t-test, $p = 0.62$, Fig. 2C). The average rate of lever presses is displayed in Fig. 2D, and representative examples of choice behavior of individual rats are shown in Fig. 2E.

Experiment 2: muscimol inactivation

Placement of the cannulae

A representative coronal brain section showing cannulae placement is shown in Fig. 3A. Cannula placements were visually verified by the location of the cannula tips. Five rats were excluded from behavioral analysis due to incorrect placement or loss of implants in the course of experiments (final $n = 7$). Fig. 3B shows a schematic illustration of all placements.

Lever press reversal

Following the completion of experiments we also tested whether the rats could reverse their choice behavior if the risks associated with the levers is reversed. As shown in Fig. 4, when the levers were switched, rats also reversed their preference, switching to the safe lever once again. There was no main effect of lever or of time ($F_s < 1$), but a significant interaction between lever and time ($F_{7, 84} = 25.7$, $p < 0.0001$). Post hoc analyses showed that choice of the left lever (now safe) increased over time, whereas the choice of the right lever decreased dramatically ($p_s < 0.0001$), resulting in a complete reversal of the choice behavior. This result demonstrate that rats were able to reverse their behavior according to the level of risk associated with each choice.

Effect of muscimol in pPPN on choice behavior under risk

Rats were tested on the choice trial task following microinjections of either control PBS or muscimol. After PBS injections, they preferred the safe lever (Fig. 5). When rats were injected with muscimol, however, risk aversion was extinguished. 2-way ANOVA with Lever and Drug as factors confirmed that there was a main effect of Lever ($F_{1,12} = 9.9$, $P < 0.01$), and, no main effect of Drug ($F_{1,12} < 1$), and significant interaction ($F_{1,12} = 17.8$, $P < 0.01$). With muscimol inactivation, the overall number of pellets earned was reduced (paired t-test, $p < 0.05$, Fig. 5B). Means rates of lever presses are shown in Fig. 5C. Individual examples of choice behavior are shown in Fig. 5D.

Discussion

In studying risk-based decision making, it is critical to manipulate specifically the level of risk, i.e. *to keep the mean reward constant while changing only the variance*. Otherwise the amount of reward is confounded with risk, making it impossible to interpret the data. In this study, the mean reward rate associated with the two levers was kept constant; the risky option and the safe option differ only in reward variance. We found that, just like mice (Leblond et al., 2011), hungry rats were also very risk averse when the reward variance is high. But the risk aversion was abolished after pPPN lesions, which reduced safe choices without affecting risky choices (Fig. 2).

Instrumental learning

The lesioned rats could still learn to press a lever for food rewards, showing no deficit in initial acquisition (Fig. 1). Both the rate of lever pressing and of head entries into the food magazine were unaffected by pre-training lesions. This pattern of results rules out gross sensory or motor deficits in the lesioned rats.

Previous work showed impaired acquisition of lever pressing after pPPN lesions (Wilson et al., 2009). But in that study the lesions were created with ibotenic acid rather than NMDA, as we did here, and the lesions were generally larger than ours, which could account for the disparity in the observations. Moreover, the deficits observed by Wilson et al. were also restricted to the initial phase of acquisition: their lesioned rats were eventually able to acquire lever pressing in the end.

As the rate of lever pressing is not an adequate measure of learning (Dickinson, 1994; Yin et al., 2005; Yin et al., 2008), the lack of any significant difference in this measure does not rule out a role for the pPPN in the acquisition of goal-directed actions. In Experiment 1, the excitotoxic lesions were created before training. Consequently we cannot rule out the possibility that pPPN lesions affected the learning of associations between action and reward magnitude. To address this problem, in Experiment 2 we used muscimol to assess the effect of reversible inactivation in rats showing strong risk aversion. As predicted, muscimol inactivation reversibly abolished risk aversion (Fig. 5).

A recent study (MacLaren et al., 2013) found that inactivation of the pPPN did not significantly impair the rate of lever pressing, but reduced the ability of the animals to reduce pressing when the action-outcome contingency was degraded by the presentation of

free rewards. The authors concluded that the pPPN is critical for updating associations between actions and outcomes. In instrumental contingency degradation, the presentation of non-contingent rewards can gradually increase competing reward seeking behavior, as animals gradually prefer the immediate freely presented reward, while reducing instrumental performance. This too is akin to a preference for exploitation.

In our study we did not change the instrumental contingency. The action-outcome relationship was maintained throughout training to achieve stable choice behavior. Our results show that when the pPPN was lesioned or inactivated, the animal drastically reduces choice of the safe lever, showing indifference between the safe and risky choice.

Alternative explanations

It is possible that differences in sensitivity to reward magnitude can account for differences in choice behavior (Locey and Dallery, 2011). That is to say, the lesion may have affected the rat's ability to distinguish between 8 pellets and 1 pellet. But this possibility is unlikely since the total number of choices and rewards earned per session remained highly stable. The lesioned rats still consumed the same amount of food in the session (Fig. 2B). Lesions of the pPPN only changed the *relative distribution of choices* on the two levers. In addition, in Experiment 2, the main effect of muscimol inactivation was a reduction in the number of safe choices (Fig. 5). The number of risky choice was not affected. This pattern suggests that disrupting pPPN function primarily reduced choice of the immediate small reward, rather than impairing the ability to discriminate between differently sized rewards.

Another possibility is that, after pPPN lesions, the rats were simply indifferent, and responded randomly. But this interpretation is not supported by our data. In particular, the data from individual rats do not show that they are distributing their choice behavior equally across two levers (Fig. 2). In Experiment 2, the more selective reduction in safe choices is also difficult to explain by random choice of levers.

Mesolimbic dopamine system and choice behavior

In our experiments, the overall payoff associated with each lever is always the same. Choice of the safe lever, or exploitation, is associated with immediate gratification. By contrast, choice of the risky lever is associated with frequent reward omissions, and delayed gratification. Such behavior is exploratory: it does not result in instant gratification, but could potentially yield larger rewards. Choice behavior under risk may therefore reflect the operation of distinct neural systems, one for the control of short-term reward and another for the control of long-term reward (Leblond et al., 2011). Our results suggest that the pPPN is a part of the neural circuit for the control of immediate rewards.

The more anterior pedunculopontine tegmental nucleus (aPPN) targets the SNc DA neurons (Pan and Hyland, 2005; Alderson et al., 2008), whereas the pPPN projects to the VTA dopamine neurons that are part of the mesolimbic dopamine system. Functionally, too, these two regions can be dissociated, as the aPPN, a potential target for deep brain stimulation treatment for Parkinson's disease, appears to be more critical for motor control, in accord with its projections to the nigrostriatal dopamine system (Alderson et al., 2008). It should be

noted, however, that the nigrostriatal dopamine system is also modulated by motivation state and a site of self stimulation. Without further experiments it would be premature to rule out a role for the aPPN in decision making under risk (Rossi et al., 2013a; Rossi et al., 2013b).

Previous work has implicated the mesolimbic dopamine system and associated circuitry in choice behavior (Winstanley et al., 2006; Salamone et al., 2009; St Onge et al., 2011). On account of its projections, lesion or inactivation of the pPPN is expected to reduce the excitatory drive to dopaminergic neurons in the VTA. The mesolimbic system has long been implicated in reward seeking (Berridge, 2007; Salamone et al., 2009; Flagel et al., 2010). Human neuroimaging studies have found ventral striatal activation that is correlated with the level of risk (Preuschoff et al., 2006; Niv et al., 2012). The available evidence from humans (Niv et al., 2012) and rodents (Sugam et al., 2012) therefore implicates the mesolimbic projection in both reward seeking behavior and risk sensitivity.

One exception comes from previous lesion work (Cardinal and Howes, 2005) showing that excitotoxic lesions of the nucleus accumbens core increased choice of “exploitation” (greater choice of immediate rewards and risk aversion). A key difference in the experimental design is that in the earlier study the overall rate of reward was not the same for the two choices. Thus, the difference in overall payoff can be confounding, making it impossible to interpret the observed changes in choice behavior as a result of lesions.

Such limitations in experimental design are also found in more recent studies on risk-related behaviors (St Onge et al., 2011; St Onge et al., 2012). To our knowledge, the present study is the first in which the rate of reward was comparable for the two choices, so that only the level risk was varied. This is a critical feature of the design that makes it possible to examine the effects of neural manipulations on decision making under risk.

Since projections from the pPPN can excite VTA dopamine neurons, our results suggest that it is part of a neural circuit responsible for the seeking of immediate, rather than delayed, rewards. This working hypothesis generates a number of predictions. Disrupting the short-term control system, by reducing pPPN projections to the dopamine neurons, results in increased reliance on the alternative risk-insensitive control system for long-term rewards. Animals might consequently become less sensitive to reward omissions. Stimulation of the pPPN or the downstream targets in the mesolimbic dopamine system is expected to potentiate the short-term control system, increasing risk aversion and exploitation of the safe option. Such predictions can be tested in future studies.

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Figure 1. Excitotoxic lesions of pPPN did not impair acquisition of acquisition of lever pressing

A. Photomicrographs showing representative excitotoxic lesions in the pPPN.

B. Illustration of the extent of lesions in all rats. Grey areas indicate the largest lesions and black areas indicate the smallest lesions. Numbers indicate distance in mm from the bregma.

C. Rate of lever pressing during initial acquisition. All rats were trained to press a lever for food reward on a continuous reinforcement (CRF, one pellet per press) followed by a fixed ratio 5 schedule of reinforcement (FR5, one pellet every 5 presses).

D. Rate of head entries into the food magazine during initial acquisition.

Figure 2. pPPN lesions dramatically reduced number of safe choices and increased number of risky choices

A. Illustration of the discrete choice operant task. Illumination of the chamber and insertion of both levers signaled the start of the trial. Choosing the safe lever always resulted in an immediate and constant reinforcement (1 pellet). Choosing the risky lever resulted in a more variable outcome, while maintaining the same overall payoff (0.125 probability of 8 pellets). Following each choice, both levers were retracted and the light was turned off. The inter-trial interval (ITI) was 40 seconds.

B. The total number of pellets earned (average last 3 sessions) was not affected by the lesion.

C. Rats with sham control lesions (Control) showed a strong preference for the safe lever, but rats with pPPN lesions did not. Risk aversion was abolished in lesioned rats.

D. Average rate of lever presses in controls and lesioned rats during the last training session.

E. Individual examples of choice behavior during the last training session. Each graph displays choice behavior of a single rat.

Figure 3. Inactivation of pPPN with muscimol abolished risk aversion

A. Photomicrograph showing representative bilateral cannulae placement in the pPPN (-8.16mm relative to bregma).

B. Illustration of the cannulae locations. Each black circle indicates the place of a chronically implanted cannula.

Figure 4. Reversal of choice behavior

Rats were able to reverse their choice behavior on the levers. Right lever was the safe lever, but became the risky lever following a reversal in the risk associated with the choices. The choice behavior also shifted accordingly.

Figure 5. Muscimol inactivation of pPPN reversibly abolished risk aversion

A. The same animals were tested on 3 daily sessions (PBS, muscimol, PBS). Rats showed a strong preference for the safe lever when injected with control PBS solution. Risk aversion was eliminated following microinjections of muscimol into the pPPN.

B. Average rate of lever presses after PBS or muscimol injection.

C. Individual behavioral data. Controls 2 and 5 are rats who received PBS injections, while Muscimol 2 and 5 are the same rats who received a muscimol injection prior to the task. The individual rats clearly showed reduced choice of the safe lever when injected with muscimol.