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Beta-adrenoreceptor blockade abolishes atomoxetine-induced risk taking

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HIGHLIGHTS

- We examined the effect of norepinephrine (NE) transmission on risk taking.
- Elevated NE level by atomoxetine increased rats' preference for risky choice.
- Elevated NE level also resulted in reduced sensitivity to losses but not to gains.
- Beta-adrenoreceptor antagonist blocked the effects of atomoxetine on risk taking.

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ABSTRACT

Rationale: Clinical studies have shown that patients with exaggerated risk-taking tendencies have high baseline levels of norepinephrine. In this work, we systemically manipulated norepinephrine levels in rats and studied their behavioral changes in a probabilistic discounting task, which is a paradigm for gauging risk taking.
Methods: This study aims to explore the effects of the selective norepinephrine reuptake inhibitor (atomoxetine at doses of 0.6, 1.0 and 1.8 mg/kg), and receptor selective antagonists (propranolol at a single dose of 1.0/kg, and prazosin at a single dose of 0.1 mg/kg), on risk taking using a probabilistic discounting task. In this task, there were two levers available to rats: pressing the 'small/certain' lever guaranteed a single food pellet, and pressing the 'large/risky' lever yielded either four pellets or none. The probability of receiving four food pellets decreased across the four experimental blocks from 100% to 12.5%.
Results: Atomoxetine increased the tendency to choose the large/risky lever. It significantly reduced the lose-shift effect (i.e. pressing a different lever after losing a trial), but did not affect the win-stay effect (i.e. pressing the same lever after winning a trial). Furthermore, co-administration of beta-adrenoreceptor antagonist, propranolol, eliminated the effects of atomoxetine on risk taking and the lose-shift effect; but co-administration of alpha$_1$-adrenoreceptor antagonist, prazosin, did not.
Conclusions: Atomoxetine boosted NE levels and increased risk taking. This was because atomoxetine decreased rats' sensitivity to losses. These effects were likely mediated by beta-adrenoreceptor.

1. Introduction

Risk taking is characterized by behaviors conducted under uncertainty with or without effective contingency planning [1]. These behaviors may harm the self or others and result in negative consequences. For example, risk-taking behavior is positively correlated with physical injuries [2]. Excessive risk taking is a symptom common to many psychiatric disorders, including pathological gambling, bipolar disorder (manic phase), substance abuse and attention-deficit/hyperactivity disorder (ADHD) [3,4,5,6,7]. Therefore, mental health professionals advocate that the awareness of potentially risky contexts and the ability to avoid severe risks are valuable skills for one to develop [8].

At the neurochemical level, it has been suggested that the tendency to take risks was correlated with norepinephrine (NE) levels in gamblers. For instance, pathological gamblers had higher baseline levels of NE and its metabolite 3-methoxy-4-hydroxyphenylglycol than normal controls [6]. In a casino gambling study, problem gamblers showed higher activation of the HPA-axis and the sympathoadrenergic system than normal controls [9].

However, behavioral research involving healthy human participants yielded inconsistent results about NE's effects on risk taking. On the one hand, O'Carroll and Papps found that NE reuptake inhibitor reboxetine did not significantly alter risky behaviors measured by performances in the Iowa Gambling Task (IGT) [10]. On the other hand, Rogers and

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colleagues used a gambling task and showed that propranolol significantly reduced healthy participants' ability to discriminate large possible losses from small ones [11]. Furthermore, elevated NE level led to significantly stronger emotional or arousal responses to losses as compared to gains [12]. These contradicting results might be caused by the experimental tasks that the researchers selected to assess risk taking under drug influences. For instance, the IGT in O'Carroll and Papps's study emphasized on gains as participants gained money on every trial but rarely lost money [13], but the gambling task in Rogers et al. emphasized on losses as participants either accepted a loss or gambled to avoid this loss (has a chance to lose more) in "losses only trials". Therefore, a plausible explanation for their disparate findings might be that altering NE only affected individuals' sensitivity to losses but not to gains, causing an overall change in observed behaviors in tasks that emphasized on losses.

In the current study, we test the above hypothesis and investigate the effects of NE transmissions on risk taking in rats. Specifically, we manipulated the NE transmissions in well-trained rats using a probabilistic discounting task, where rats chose between a small/certain reward and a large/risky reward. The drugs used to manipulate NE transmissions were: selective NE reuptake inhibitor atomoxetine, α_1 adrenergic receptor antagonist prazosin, and β adrenergic receptor antagonist propranolol. Moreover, we analyzed the effect of the win/lose outcome of a given trial on choice behavior of the next trial. In other words, we closely examined whether winning a bet would make the rats keep pressing the same lever (win-stay effect), and whether losing a bet would make the rats press the alternative lever (lose-shift effect) on a subsequent trial. The win-stay effect was used as an index for sensitivity to rewards and the lose-shift effect served as an index for sensitivity to negative feedbacks.

2. Materials and methods

2.1. Subjects

Twelve male Sprague Dawley rats weighing 250–280 g right before training were used. Ten of them met the inclusion criteria after training (see below "*Inclusion Criteria*") and hence completed the drug manipulations. All rats were separately housed under regular lighting condition (lights on from 8:00 AM to 8:00 PM). During the entire duration of the experiment, rats had access to water ad lib and they were fed daily in their home cages at night. We monitored rats' weights daily and maintained their weights at 85–90% of their free feeding weights. All procedures were in accordance with the National Institutes of Health Guide (revised 1996) for the Care and Use of Laboratory Animals.

2.2. Behavioral apparatus

Behavioral testing for all experiments was conducted in four identical operant chambers (29 × 29 × 26 cm; Anilab Software and Instruments Co., Ltd., Ningbo, Zhejiang, China) each enclosed in a sound-attenuating box. Each box was equipped with a fan to provide air circulation. Chambers were fitted with a 100-mA overhead house light and two retractable levers located on either side of a central alcove, where sugary food reward pellets (45 mg; Research Diets, New Brunswick, NJ) were delivered by an automatic dispenser. Apparatus control and data recording were performed using Anlilab software version 4.34 (Anilab Software and Instruments Co., Ltd., Ningbo, Zhejiang, China).

2.3. Initial training

All rats were trained to press levers according to the following protocol. Prior to their first training day, each rat received 25 food pellets in its home cage. In the first training session, crushed pellets were placed on two levers and 4 pellets were delivered into food alcove. First, rats were trained to press one lever (counterbalanced to press the left or right lever first) for a single pellet on a fixed-ratio-1 schedule, until they press no less than 60 times in 30 min.

Afterwards, the rats were familiarized with the probabilistic nature of the experimental task. This part contained 90 trials. At the beginning of each trial, the operant chamber was kept dark and the levers were retracted. A trial started with the house light turned on and a lever extended out (randomized left or right lever). If the rat failed to press the lever within 10s, the house light was turned off, and the trial was recorded as an omission. If the rat responded in time, the lever retracted and one pellet was delivered with a 50% probability. The rats were trained for 7–10 days until they reliably pressed the levers in 80 out of 90 trials.

2.4. Probabilistic discounting task

In this experiment, we used a probabilistic discounting task (PDT) adapted from Cardinal and Howes and St Onge, et al. to study NE's effect on risky behaviors [14,15], see Fig. 1. Rats were trained for 5–7 days a week. On each day, rats completed one training session that consisted four blocks. Within each block, the probability of winning was constant, and across blocks, it systematically decreased from 100% to 50%, to 25%, and finally to 12.5%. In each block, there were eight forced-choice trials and ten free-choice trials. On a given forced-choice trial, only one lever was presented. Across the 8 forced-choice trials, left and right levers each occurred four times in random orders. The forced choice trials were to make rats learn the winning probabilities in that block. These were followed by ten free-choice trials, where both levers were present and the rats had to choose one. For both forced-choice and free-choice trials, the chamber was kept dark with both levers retracted before trials began. A trial began when the house light was turned on. After 3 s, the lever(s) extended into the chamber. For each rat, one lever was the large/risky lever (which remains consistent throughout training, counterbalanced left/right), pressing which would yield four food pallets but with varied probabilities; the other lever was the small/certain lever, pressing which would yield one food pellet at 100% probability. Once a lever was pressed, the lever(s) retracted immediately and food pellet(s) were delivered into the alcove. If pellet(s) were delivered, the house light remained on for additional 4 s before it shut off, which marked the end of a trial. If no pellet was delivered after a press or rats did not press any lever in 10 s (which would be considered as an omission), the trial ended and the house light was turned off. The next trial began after 15 s (i.e. the inter-trial intervals were 15 s).

2.5. Inclusion criteria

Rats were trained on the task until they met the following criteria: (1) in the first block, where the probability of winning a risky bet was 100%, rats chose the large/risky lever for more than 8 times in 10 trials; (2) in the fourth block, where the probability of winning a risky bet was 12.5%, rats chose the large/risky lever for less than 6 times in 10 trials; and (3) in three consecutive training days, rats showed stable baseline patterns of choice, that is, their choice behaviors differed significantly between blocks that had different winning probabilities. Reflected statistically, this means rats' choice behaviors were significantly affected by the factor of Block but not affected by Day or Day × Block interaction.

Ten out of 12 rats met all three criteria after 16–22 days of training and hence proceeded to receive drug treatments. Two rats were excluded from the experiment because they did not meet the second and third criteria.

2.6. Drug manipulations

Atomoxetine and two kinds of adrenoreceptor antagonists (propranolol and prazosin) were administered. Three doses of atomoxetine, 0.6, 1.0 and 1.8 mg/kg (DingHui chemical Co Ltd., Wuhan, China) were dissolved in 0.01 M phosphate-buffered saline (PBS). We chose to inject

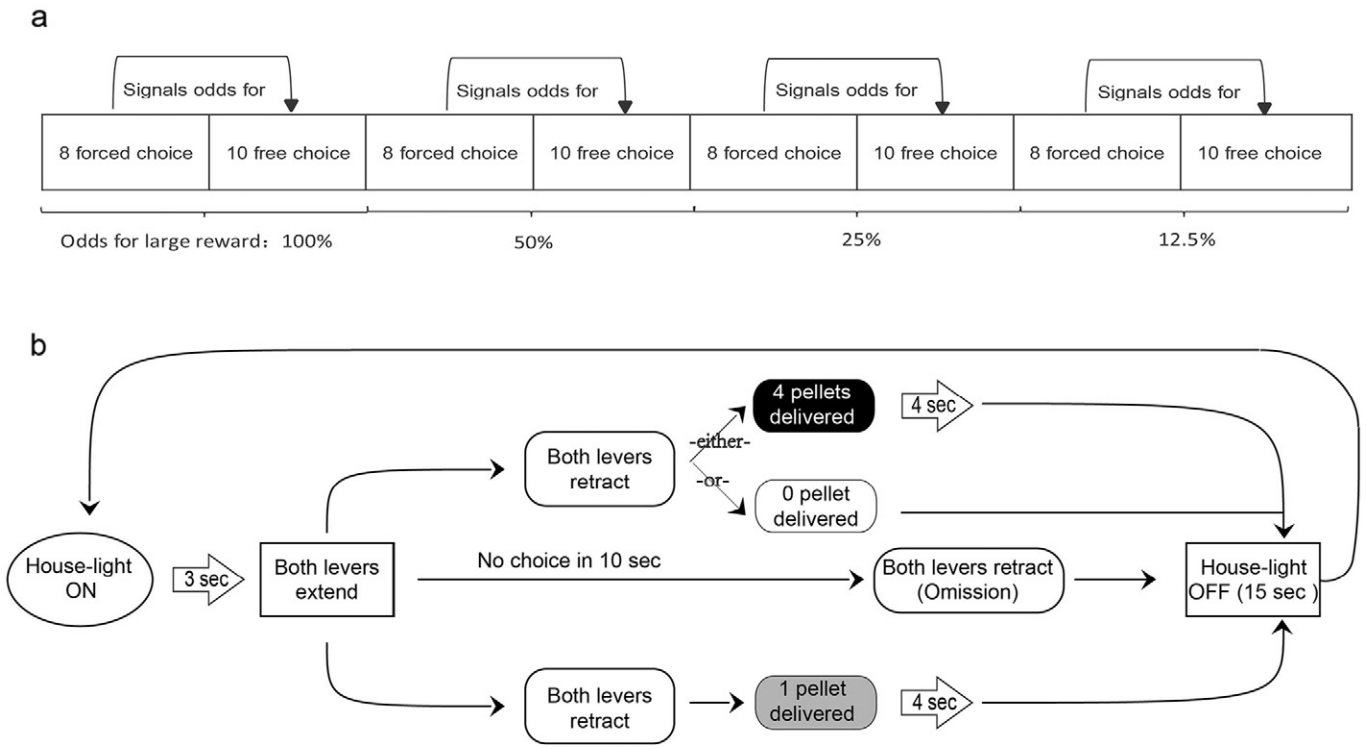

Fig. 1. Schematic drawing of the PDT. a, Arrangement of experimental blocks and trials. b, Procedure of the free choice trials. This picture was adapted from St Onge et al. with modifications [15].

atomoxetine at these three doses because previous studies reported that these levels of atomoxetine affected cognitive functioning and impulsivity [16,17,18]. Adrenergic α_1 receptor antagonist prazosin (0.1 mg/kg, Sigma-Aldrich, Shanghai, China) and β receptor antagonist propranolol (1 mg/kg, Sigma-Aldrich, Shanghai, China) were diluted in sterile saline. These two doses were selected because previous studies showed that at these dosage levels, the drugs had no effect on attention [19].

All drugs were administered to each rat in the following steps: first, different doses of atomoxetine or the vehicle; second, propranolol or the vehicle or propranolol and atomoxetine together; third, prazosin or the vehicle or prazosin and atomoxetine together, see Table 1 for drug manipulation summary. Different drugs (including different doses of same drug) in each step were injected in separate day according to a Latin Square design. Drugs were prepared fresh every day and were injected intraperitoneally at 30 min (prazosin and propranolol) or 40 min (atomoxetine) before testing with the volume being 1 ml/kg. Rats completed one session of the PDT (4 blocks) after each drug injection and their performance was recorded. Between every two tests, there were at least two washout days for the effect of previously injected drugs to wear out and the rats had to achieve stable baseline choice behavior patterns before they could move on to the next drug.

Table 1
Experiments performed.

Sequence	Manipulation	Dose (mg/kg)
Step 1	Vehicle	PBS + saline
	Atomoxetine + saline	0.6 + saline
		1.0 + saline
		1.8 + saline
Step 2	PBS + propranolol	PBS + 1
	Vehicle	PBS + saline
	Atomoxetine + propranolol	1.8 + 1.0
Step 3	PBS + prazosin	PBS + 0.1
	Vehicle	PBS + saline
	Atomoxetine + prazosin	1.8 + 0.1

2.7. Data analysis

The primary outcome measure was how many times rats pressed the large/risky lever in the free-choice trials in the four blocks with different winning odds and under the influences of various drugs. Choice behavior data first went through an arcsin transformation to avoid the ceiling effect that rats chose the large/risky lever in all trials in the first block with 100% winning probability [20]. Transformed choice behavior data was analyzed using two-way repeated measures ANOVA with Block (4 levels of winning probabilities) and Drug Type (which includes 4 drug doses in Step 1 and 4 different drug types in Step 2 and 3) as independent variables. In each of these analyses, the main effect of Block was always significant ($p < 0.001$), which indicated rats were well aware of the probabilistic nature of the current task. This will not be reported further.

In addition, we analyzed, in separate univariate 1-way repeated measures ANOVAs, how Drug Type affected trial omissions, number of total pellets obtained, and response latencies.

We also investigated whether knowledge of previous choice outcomes affected current-trial behaviors. After winning a large/risky bet, if a rat continued to press the large/risky lever, there was a win-stay effect. After losing a large risky bet, if the rat pressed the alternative lever on the next trial, there was the lose-shift effect. Because norepinephrinergic system might be related to individuals' sensitivity to losses but not to gains, when atomoxetine was administered, we expected to see a changed lose-shift effect but an unchanged win-stay effect in rats' behavior. We looked at performances on consecutive trials and computed the ratio of number of trials with win-stay effect over number of trials when rats won a large/risky bet, as well as the ratio of number of trials with lose-shift effect over number of trials when rats lost a large/risky bet. This was repeated for each rat's performance in all blocks for all drug manipulations. We

analyzed how these ratios were affected by drug manipulation using a repeated measures ANOVA.

3. Results

3.1. Effects of atomoxetine on PDT performance

Atomoxetine significantly increased choice of the large/risky lever ($F(3, 27) = 4.422$, $p = 0.012$, $\eta_p^2 = 0.329$). The Block × Drug Type interaction also reached statistical significance ($F(9, 81) = 2.062$, $p = 0.043$, $\eta_p^2 = 0.186$). Further analysis showed that, comparing to the vehicle, atomoxetine significantly increased pressing the large/risky lever in the 50% winning probability block ($F(3, 7) = 4.935$, $p = 0.038$, $\eta_p^2 = 0.679$) and in the 25% winning probability block ($F(3, 7) = 4.658$, $p = 0.043$, $\eta_p^2 = 0.666$), see Fig. 2a. Because the 1.8 mg/kg dose of atomoxetine produced the largest increase in risky choice, this dose was used in subsequent drug combinations with adrenoreceptor antagonists, see Fig. 2a.

Treatment with atomoxetine significantly increased response latencies ($F(3, 27) = 3.912$, $p = 0.019$, $\eta_p^2 = 0.303$), see Table 2. Post-hoc analysis showed that only the 1.8 mg/kg dose of atomoxetine significantly increased response latencies ($p = 0.011$).

The lose-shift effect ($F(3, 27) = 6.058$, $p = 0.003$, $\eta_p^2 = 0.402$) but not the win-stay effect ($F(3, 27) = 0.331$, $p = 0.803$) was significantly reduced by atomoxetine. Post-hoc analysis showed that all three doses of atomoxetine significantly reduced lost-shift effect, as compared to the vehicle ($p < 0.05$, in all post-hoc tests), see Fig. 2b.

In addition, during the entire study, none of the drug types significantly changed the number of total pellets obtained ($F(9, 81) = 0.502$, $p = 0.869$) and the number of omissions ($F(9, 72) = 0.981$, $p = 0.463$), which suggested that rats' motivation for eating did not change during the experiment. This will not be reported further, see Table 2.

3.2. Beta-adrenoreceptor antagonist propranolol

We analyzed how beta-adrenoreceptor blockades (with propranolol alone and with the combination of propranolol and atomoxetine) affected rat's behaviors in this study, with risky choice, response latency and lose-shift/win-stay patterns as outcome variables. First, propranolol successfully reduced the atomoxetine-induced increase in risky choice, see Fig. 3a. Analysis of the choice data revealed a significant main effect of Drug Type ($F(3, 27) = 5.705$, $p = 0.004$, $\eta_p^2 = 0.388$). Post-hoc analysis showed that risky behaviors increased more with atomoxetine treatment than with the vehicle, with propranolol and with the drug combination of propranolol and atomoxetine (hereinafter, drug combination) ($p < 0.05$, in all cases). The Block × Drug Type interaction was not significant ($F(9, 81) = 0.76$, $p = 0.654$).

Response latency data was affected by Drug Type ($F(3, 27) = 3.211$, $p = 0.039$, $\eta_p^2 = 0.263$). Comparing to the vehicle, both drug combination ($p = 0.034$) and atomoxetine ($p = 0.004$) significantly increased response latencies, see Table 2.

The effect of Drug Type on lose-shift effect was significant ($F(3, 7) = 4.659$, $p = 0.043$, $\eta_p^2 = 0.666$). Comparing to the vehicle, only atomoxetine ($p = 0.009$) but not propranolol ($p = 0.608$) or drug combination ($p = 0.639$) attenuated the lose-shift effect. Therefore, co-administration of propranolol decreased the effect of atomoxetine on lose-shift behaviors. The effect of Drug Type on the win-stay effect did not reach statistical significance ($F(3, 27) = 0.374$, $p = 0.772$), see Fig. 3b.

Table 2
Response latency, pellets obtained, trial omissions for drug treatments.

Treatments	Latency	Pellets obtained	Omissions
Step 1			
Vehicle	1.66 ± 0.08	119 ± 1.81	0.1 ± 0.1
Atom 0.6 + saline	1.78 ± 0.07	122.1 ± 1.83	0.1 ± 0.1
Atom 1.0 + saline	1.81 ± 0.11	120.7 ± 2.01	0.7 ± 0.43
Atom 1.8 + saline	1.88 ± 0.10*	120.5 ± 1.63	0.5 ± 0.31
Step 2			
PBS + pro 1.0	1.82 ± 0.09	119.5 ± 1.82	0.1 ± 0.1
Vehicle	1.742 ± 0.12	118.4 ± 2.67	0.1 ± 0.1
Atom 1.8 + pro 1.0	1.89 ± 0.08*	119.8 ± 1.66	0
Prazosin			
PBS + pra 0.1	1.92 ± 0.14	122.1 ± 1.64	0.1 ± 0.1
Vehicle	1.83 ± 0.12	120.1 ± 1.05	0.4 ± 0.16
Atom1.8 + pra 0.1	2.12 ± 0.12*	122.1 ± 2.01	0.4 ± 0.31

* $p < 0.05$ vs vehicle.

3.3. Alpha$_1$-adrenoreceptor antagonist prazosin

We analyzed how prazosin, atomoxetine and their combination affected rat's behaviors in terms of risky choice, response latency and lose-shift/win-stay patterns. Risky choice was marginally affected by Drug Type (the four levels of this variable were: the vehicle, prazosin, atomoxetine and the drug combination of prazosin and atomoxetine) ($F(3, 27) = 2.738$, $p = 0.063$, $\eta_p^2 = 0.233$). Treatment with atomoxetine

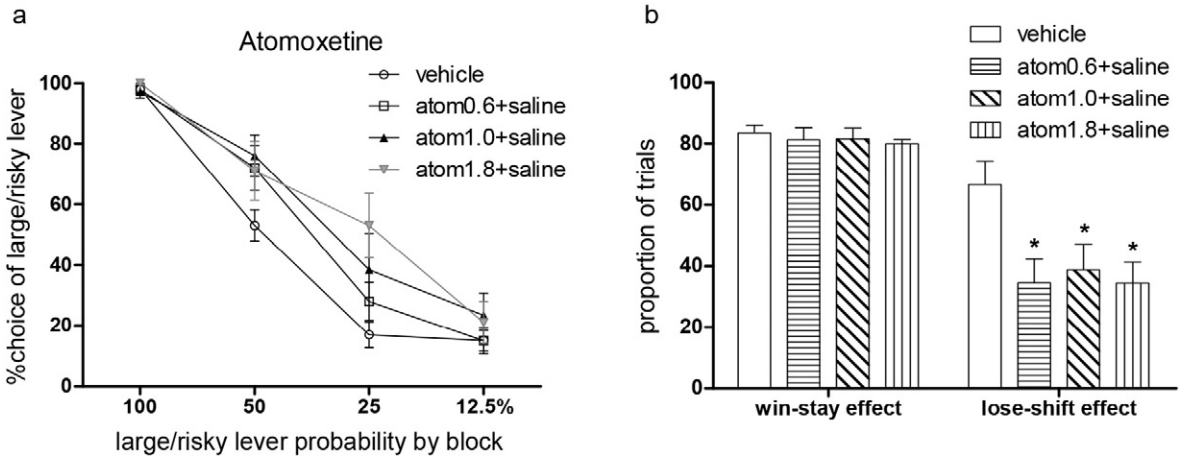

Fig. 2. The effects of atom (atomoxetine) on PDT performance. (a) Atomoxetine increased the preference to large/risky rewards. Treatment with atomoxetine significantly increased risky choice on the 50% and 25% trial blocks as compared to the vehicle ($p < 0.05$). (b) All doses of atomoxetine significantly reduced sensitivity to negative feedback in rats.*$p < 0.05$ vs vehicle.

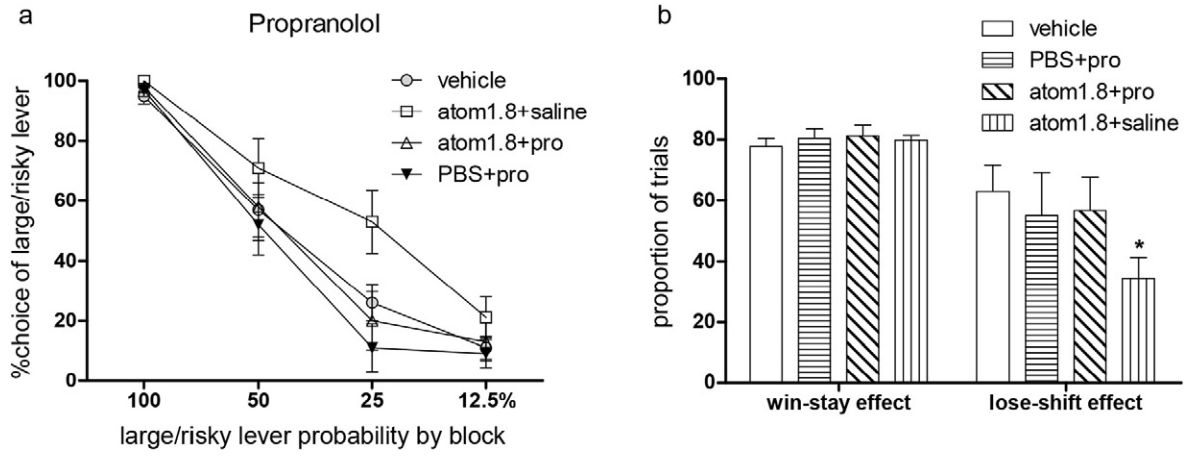

Fig. 3. The effects of combinations of the 1 mg/kg dose of pro (propranolol) and the 1.8 mg/kg dose of atom (atomoxetine). (a) Propranolol completely blocked atomoxetine-induced risk preference. Treatment with atomoxetine alone significantly increased risky choice as compared to the other three groups ($p < 0.05$, in all cases). (b) Only treatment with atomoxetine alone significantly decreased the lose-shift effect as compared to the vehicle. *$p < 0.05$ vs vehicle.

significantly increased risky choice than the vehicle ($p = 0.045$). Co-administration of prazosin did not alter the existing effect of atomoxetine on risky choice ($p = 0.524$). The Block × Drug Type interaction on risky choice was not significant ($F(9, 81) = 1.454$, $p = 0.179$), see Fig. 4a.

Response latency was significantly affected by Drug Type ($F(3, 27) = 3.728$, $p = 0.023$, $\eta_p^2 = 0.293$). The drug combination of prazosin and atomoxetine significantly increased response latencies as compared to the vehicle ($p = 0.029$), see Table 2.

Both treatments with atomoxetine and with the combination of prazosin and atomoxetine significantly reduced the lose-shift effect, as compared to the vehicle ($F(3, 27) = 3.034$, $p = 0.046$, $\eta_p^2 = 0.252$), but neither affected the win-stay effect ($F(3, 27) = 1.043$, $p = 0.39$).

4. Discussion

In this work, we investigated how NE affected risk taking. First, increased NE level in rats yielded increased risk-taking behaviors. In this study, administration of atomoxetine, which increased NE, significantly increased risky choices made by rats, and this was accompanied with reduced lose-shift behaviors. These effects of atomoxetine on risk-taking behaviors were blocked by β receptor antagonist propranolol; but they were largely unaffected by α_1 receptor antagonist prazosin.

4.1. Atomoxetine induced risk preference

The current study examined the effects of NE on risky behaviors using the PDT, which has been relatively unexplored hitherto [21]. Results from the first step of this study showed that when atomoxetine was administered, rats made risky choices more frequently. This is because atomoxetine increased the NE level, and excessive NE promoted risky behaviors by reducing sensitivity to losses. Because atomoxetine increases both NE and dopamine (DA) release in the prefrontal cortex (PFC) [22], and DA transmission mediates risk-taking behaviors [12, 23], one could argue that it was the increased DA in PFC, but not NE, that caused an increase in seeking large/risky rewards in this experiment. Previous research [24] has found that excessive DA transmission in the PFC impaired reward processing in the PDT, and reduced rats' ability to adjust their choices according to the winning probabilities. In other words, the discounting rates would not change with changing winning probabilities and rats consistently showed preference for risky choice in all four blocks. In contrast, in the current study, rats displayed a significant discounting based on different winning odds:

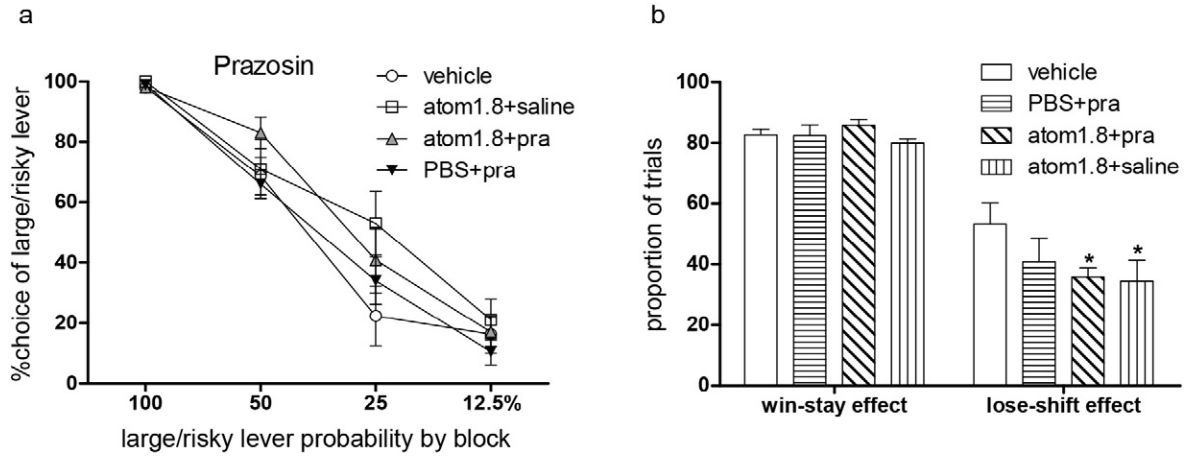

Fig. 4. The effects of combinations of the 0.1 mg/kg dose of pra (prazosin) and the 1.8 mg/kg dose of atom (atomoxetine). (a) Prazosin did not alter the effects of atomoxetine. The main effect of Drug Type approached statistical significant ($p = 0.063$). (b) Atomoxetine reduced sensitivity to negative feedback (the lose-shift effect). Prazosin did not further change this effect. *$p < 0.05$ vs vehicle.

they made more risky choices in the 100% and 50% blocks than in the 25% and 12.5% blocks. This indicated that atomoxetine-induced increase in PFC DA could not fully account for rats' behavior in the current study. Furthermore, the subsequent treatment with β-adrenoreceptor antagonist yielded results that directly supported the contribution of NE on the observed risky behaviors. When the β-adrenoreceptor antagonist was injected along with atomoxetine, β receptor antagonist reduced the NE transmission but did not change the DA transmission. In this case, we observed that propranolol completely blocked the effects of atomoxetine on risk taking. Therefore, increased NE must be the primary factor accountable for the inclination to take more risks when atomoxetine was injected. This was further verified by a detailed investigation of the trial-by-trial choice pattern.

We studied rats' choice patterns on consecutive trials to probe their sensitivities to losses and gains under the influence of atomoxetine. In the control condition, right after winning a risky choice, rats chose the same lever on about 83% of the trials; after losing a risky choice, rats shifted to the small/certain option on approximately 66% of trials. These results suggested that consequences of a risky choice exerted a powerful influence on determining which option rats would choose on the next trial. In the atomoxetine condition, the chemical decreased lose-shift effect, where after losing a risky choice rats shifted and chose the small/certain lever on 35% of the trials; but it did not affect the win-stay effect, where after winning rats chose to press the same large/risky lever in over 80% of the trials. This suggested that with increased NE, losing a large reward became less effective in leading to a more conservative, small/certain choice on the next trial. In other words, rats were less sensitive to negative feedback after atomoxetine treatment.

Results from the trial-by-trial analysis confirmed our hypothesis that altering NE only affected individuals' sensitivity to losses but not to gains. Therefore, the previously observed inconsistent results in human studies may be caused by the differences in task designs. For example, the IGT involves elements of reversal learning because the odds of winning or losing are unknown to participants [25]. Thus, probability assessments may be confounded with risk preference. More importantly, the IGT places more emphases on gains [13], i.e. participants gain money on every trial with occasionally losses. Due to these features of the IGT, elevated NE level did not alter healthy human participants' risky behaviors in this task [10]. Correspondingly, an animal research using rat model of IGT also found that increased NE level with atomoxetine injection did not change rats' risk preference [26].

In terms of neural mechanism, several studies suggested that a possible brain region involved in atomoxetine-induced risky behavior is the insula. Insular activity is positively correlated to loss aversion [27,28,29,30] and negatively correlated with NE level in plasma [31]. Therefore, the elevated NE level in the current study might result in lower insular activity (possibly via the afferent vagus nerve expressing β-adrenoreceptor), which made individuals less sensitive to losses.

Additionally, in this study rats' lever-pressing response latencies were increased under the influence of atomoxetine. Similar effects were found in other types of discounting tasks [16,18]. We speculate that with reduced sensitivity to negative outcomes, it might be more difficult for rats to make a choice between the given options, hence leading to longer response latencies.

4.2. Beta-adrenoreceptor, but not alpha$_1$-adrenoreceptor, plays a critical role on risk taking

Results from the current study showed that the β-adrenoreceptor antagonist propranolol (at a single dose of 1.0 mg/kg) alone did not affect risky choice, but it blocked the effects of atomoxetine on risk taking and the sensitivity to losses. In contrast, the α_1-adrenoreceptor antagonist prazosin (at a single dose of 0.1 mg/kg) had minimal effects on atomoxetine-induced risk preference. These dissociable effects of α- and β-adrenoreceptor were congruent with results from impulsivity studies (measured by the Five-Choice Serial Reaction Time task). Milstein et al. found that prazosin partially reduced methylphenidate-induced premature responding, while propranolol completely eliminated methylphenidate-induced impulsivity [19]. Relatedly, Liu and colleagues showed that prazosin reversed the reboxetine-mediated increment of response accuracy while α_2-adrenoreceptor antagonist RX821002 abolished the reboxetine reduced impulsivity [32]. Furthermore, treatment with NE α_2-adrenoreceptor antagonist clonidine reduced risk taking in abstinent heroin abusers [33]. In sum, these findings and results from the current study suggest that α_1-adrenoreceptor is likely to be involved in attentional processing (in terms of premature responding and response accuracy), while α_2- and β-adrenoreceptor might contribute to the adjustment of impulsivity and risk taking.

4.3. Three reasons for behavioral changes in the PDT

Generally speaking, there are three reasons that could explain rats' behavioral changes in the PDT. First, the appreciation of reinforcers' values might be impaired. For example, inactivation of nucleus accumbens reduced the preference for larger rewards even when the wining probability was 100% [34]. Second, the ability to update reinforcers' values might be hindered. For example, medial PFC inactivation resulted in perseveration, where rats constantly favored the lever that they pressed at the beginning of a session. In other words, rats with medial PFC inactivation did not alter their choice based on the changed winning probabilities [35]. Third, the inclination to take risks might be changed. For example, when the basolateral amygdala was lesioned, Ghods-Sharifi, St Onge and Floresco observed that rats behaved less riskily when the winning probabilities were 50% and 25% but not when the winning probabilities were 100% or 12.5% [36]. The winning probabilities' effect on choice behaviors was not uniform across the board, which suggested that rats were able to perceive, differentiate and update the winning probabilities. Therefore, ruling out reasons 1 and 2, the only reason that could explain rats' behavior changes in Ghods-Sharifi, St Onge and Floresco's study was that rats became more willing to take risks when the winning probability was neither too high (100%) nor too low (12.5%).

Similarly, results of the current study could be explained by the third reason, but not the first or second reason as previously listed. Regarding the first reason, rats chose the large/risky lever in almost all trials with 100% winning probability, which indicated that rats were able to apprehend reinforces' values. Regarding the second reason, when the winning probability dropped to 12.5%, rats always showed less preference to the large/risky lever regardless of drug treatment, which suggested that their ability to update reinforcers' values remained unchanged, in other words, they adjusted their behaviors according to the different winning probabilities. Unlike rats with medial PFC inactivation, whether the sequence of probabilities is increasing or decreasing across four blocks would not affect the effect of drugs on rats' choice pattern in the current study. Hence, only the third reason might account for our results. Treatment with atomoxetine only increased risk preference in the 50% and 25% blocks, which suggested that rats became more willing to take risks under the influence of increased NE.

4.4. Clinical implications

Behaviorally, pathological gamblers have higher risk-taking tendencies and lower probabilistic discounting rates than normal controls [37]. Neurochemically, pathological gamblers have higher cerebrospinal fluid levels of NE metabolite 3-methoxy-4-hydroxyphenylglycol and greater urinary outputs of NE [6,38]. Our research showed that, in rats, β-adrenoreceptor antagonist propranolol (at a low dose which has no effect on attention) reduced risky behaviors that were initially induced by NE. Therefore, applying this finding to human individuals, propranolol (even at a low dose that will not affect cognitive processing) may

have a potential therapeutic role in reducing NE levels and hence lessening abnormal risk taking in pathological gamblers.

Moreover, atomoxetine is a commonly used drug for ADHD [39]. ADHD patients have higher levels of impulsivity and risk taking than normal controls [4,40]. Plenty of research has shown that atomoxetine treatment significantly reduced impulsivity in ADHD [17,41,42]. However, our results suggested that atomoxetine might increase risk taking in ADHD patients as a side effect. In fact, researches have consistently found a positive relationship between ADHD and problem gambling [43,44], which is possibly because that atomoxetine treatment of ADHD promoted problem gambling in these patients. Future studies should therefore focus on this side effect of atomoxetine treatment.

5. Conclusions

With ten trained rats and using a PDT, the present research confirmed that atomoxetine increased NE level, which increased risky behaviors particularly by reducing subjects' sensitivity to losses but not to gains. Furthermore, beta-adrenoreceptor antagonist reduced risky behaviors by restoring subjects' sensitivity to losses. These findings have implications for understanding and hence countering the increased risk taking in pathological gamblers.

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