Serotonin and Effort-Based Decision-Making: Dissociating Behavioral Effects of 8-OH-DPAT and PCPA

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Summary

Effort-based decision-making is particularly relevant to psychiatric conditions where motivation deficits are prominent features. Despite its clinical significance, the neurochemical mechanisms of this cognitive process remain unclarified. This study explores the impact of serotonin synthesis inhibition (PCPA) and modulation of serotonin release and 5-HT1A receptor agonism (8-OH-DPAT) on effort-based decision-making in rats. Adult male rats were trained in a modified T-maze task where they could obtain a high reward for climbing a mesh barrier or a low reward for no extra effort. Following training, rats received either acute 8-OH-DPAT treatment or subchronic PCPA treatment and were tested on their choices between high- and low-effort arms. The goal-arm choices and goal-arm entrance latencies were recorded. Next, homovanillic acid and 5-hydroxyindoleacetic acid, metabolites of dopamine and serotonin, respectively, were quantified in the rats' prefrontal cortex, striatum, and hippocampus. 8-OH-DPAT significantly increased low-effort, low-reward choices and increased goal-arm latency. In contrast, PCPA treatment did not affect these measures. Both PCPA and 8-OH-DPAT significantly decreased 5-hydroxyindoleacetic acid levels in the prefrontal cortex and the hippocampus. 8-OH-DPAT treatment was also associated with decreased homovanillic acid levels in the hippocampus. Our findings suggest that the overall reduction of serotonin levels alone does not affect effort-based decision-making and highlights the possible role of the hippocampus and the 5-HT1A receptor in this cognitive process.

Key words

Decision-making • Effort • Serotonin • 8-OH-DPAT • PCPA

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Introduction

Effort-based decision-making is a process by which individuals weigh the potential benefits of a reward against the costs associated with obtaining that reward, where the costs often involve physical or cognitive effort [1]. Effort-based decision-making is relevant to clinical situations where deficits of motivation are prominent features, such as depression [2,3], schizophrenia [4], Parkinson's disease [5,6], and substance abuse [7]. Dysfunctional decision-making is not only one of the core symptoms of these conditions but also complicates therapeutic interventions, often leading to early relapse [8,9] and treatment drop-out [10]. Despite its clinical importance, the neurobiological mechanisms underlying decision-making under both healthy and pathological conditions remain unclarified.

Several brain regions have been implicated in effort-based decision-making, including the prefrontal cortex (PFC), striatum, and hippocampus. The PFC, particularly its dorsolateral and ventromedial subdivisions, plays a role in integrating information about effort and reward to guide decision-making [11,12]. The striatum, especially the nucleus accumbens, is involved in effort and reward evaluation and cost-benefit analysis,

with higher activation associated with selecting high-effort, high-reward options [1,13]. The hippocampus might contribute to decision-making by encoding the value of future outcomes [14], flexible generalization of past experiences to new choices [15] and relaying memory-related information to the ventromedial PFC to facilitate the estimation of the subjective value of past experiences [16,17]. Nonetheless, while the neural basis of effort-based decision-making has been extensively investigated, the underlying neurochemical mechanisms remain insufficiently understood.

The interest in the serotonergic system in decision-making arises from observations that psychiatric disorders associated with abnormalities in serotonergic neurotransmission are often accompanied by decision-making deficits [18,21]. Previous studies have shown that manipulating the serotonergic system can influence decision-making processes; however, these have largely focused on aspects other than effort valuation, such as risk assessment and reward processing [22-25]. Their results imply that serotonin plays a role in value-based decision-making, presumably by facilitating decisions based on risk and reward assessment in tasks that involve probabilistic reward and punishment. However, the role of serotonin in effort-based decision-making remains to be elucidated.

In the present study, we investigated the role of serotonin in effort-based decision-making using two rodent pharmacological models: an acute 8-hydroxy-DPAT hydrobromide (8-OH-DPAT) treatment and a subchronic para-chlorophenylalanine (PCPA) treatment. 8-OH-DPAT affects serotonin release and signaling by acting as an agonist of somatodendritic 5-HT1A autoreceptors and postsynaptic 5-HT1A heteroreceptors located mainly on pyramidal neurons and interneurons [26]. In contrast, PCPA inhibits serotonin synthesis by irreversibly blocking tryptophan hydroxylase, a key enzyme in serotonin production. Since decreased serotonin transmission has been associated with decreased motivation, particularly reward-based motivation [27], we hypothesized that reduced serotonergic synthesis and decreased serotonergic transmission would decrease the rats' willingness to exert effort to gain higher rewards. Next, we explored treatment-associated changes in 5-hydroxyindoleacetic acid (5-HIAA) concentration, an indicator of serotonin turnover [28], in the hippocampus, PFC, and striatum. Given serotonin's modulatory effect on the release of dopamine [29], which has been previously implicated in the decision-making process [30], we also assessed levels of homovanillic acid (HVA), a dopamine metabolite, in the selected brain regions.

Materials and Methods

Animals

Male Long-Evans rats (n=32, 12 weeks) obtained from the IPHYS breeding colony (Prague, Czech Republic) were housed in pairs in the animal facility (22±1 °C, 50 % humidity, 12-hour light-dark cycle), with ad libitum access to water and rodent chow. After a 10-day acclimatization, rats were handled for five minutes daily for five days. Chow access was restricted to 85-90 % of free-feeding weight five days before the behavioral experiment onset and maintained throughout the study. One day before behavioral experiments, rats were introduced to chocolate-flavored pellets (Nesquik; Nestlé) in their home cages. During subchronic oral treatments (PCPA or vehicle), rats were housed individually to ensure each rat consumed the calculated amount of substance. The experiments were approved by the Institutional Animal Care and Use Committee (project authorization no. 79-2022-P) and complied with the Animal Protection Act of the Czech Republic, EU directive (2010/63/EU).

Apparatus

The custom-made T-maze apparatus (Fig. 1A) was constructed using dark PVC. The maze consisted of a wide central arm (16×50 cm, start arm) and two perpendicular side arms (10×50 cm, goal arms), each containing glass food wells fixed to the arena floor. Both goal arms were fitted with guillotine doors manually operated by the experimenter. During the training and the testing session, a triangular barrier crafted from metal mesh (15 cm or 30 cm tall) was firmly inserted in the goal arm, obstructing access to the high food reward (four chocolate pellets).

Effort-based decision-making task

Rats were trained and tested in a modified T-maze barrier task across 22 days (Fig. 1B). All tests were conducted during the light phase and were recorded using an overhead camera. The discrimination and barrier training trials were conducted in cycles (n=4 per group) to ensure the intertrial interval did not exceed 5 min. Unless stated otherwise, rats had to choose the goal arm and consume the reward within 150 s, or the trial was terminated.

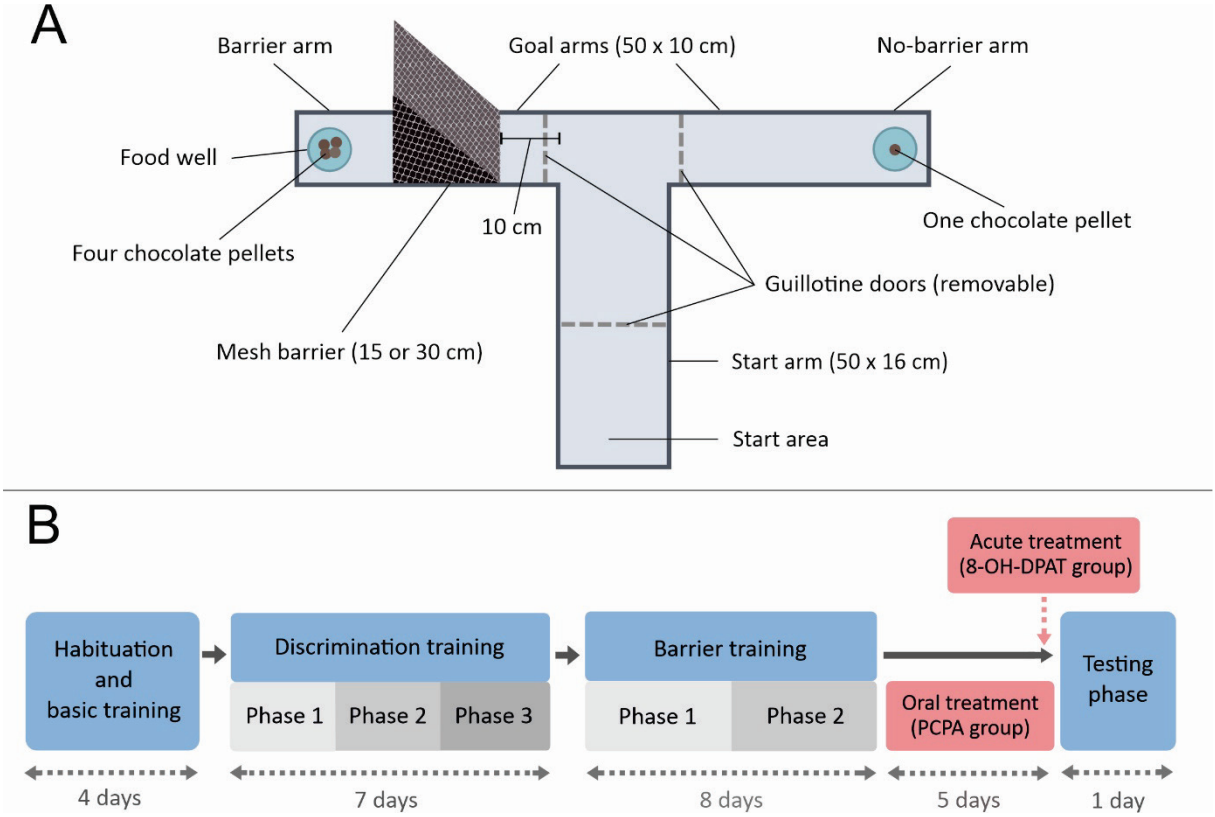

Fig. 1. The T-maze apparatus used in the effort-based decision-making task and experiment overview. (**A**) Walls (30 cm high, thick blue lines) are not displayed. The barrier arm contained four chocolate pellets, and the no-barrier arm contained a single chocolate pellet. (**B**) The overview of the key steps in the effort-based decision-making task. Following habituation, rats received basic discrimination and barrier training to learn barrier climbing and reward contingencies. Next, rats in the PCPA group received daily oral treatment with PCPA or vehicle. One day prior and on the day of the testing phase, rats in the 8-OH-DPAT group received subcutaneous treatment with 8-OH-DPAT or saline. Immediately after the testing phase, rats were sacrificed to collect brain samples.

Habituation and basic training

Rats were initially paired and habituated (15 min) to the T-maze containing eight chocolate-flavored pellets in glass wells at the end of goal arms. Next, rats underwent basic training (three daily trials for three days), requiring them to consume a single pellet from each food well within 150 s. Rats advanced to the next phase if they met the criteria during all trials on the final training day.

Discrimination training

During discrimination training, one goal arm contained a high reward (four pellets) while the other contained a low reward (one pellet). High and low reward placement was randomly chosen for each rat and counterbalanced across groups. Reward contingencies remained constant for each rat throughout the experiment. Initially, rats learned of the location of high- and low-reward arms by consuming pellets from both (five daily trials for two days). In the next phase, rats were trained to select only one reward (ten daily trials for two days). A divider blocked access to one randomly chosen goal arm, forcing rats to enter the other. To prevent side bias, no animal was forced into the same arm more than twice consecutively. The allocation of forced visits to high and low reward arms was counterbalanced within each session. The final discrimination training phase established the *first-choice rule* (ten daily trials for three days). Rats were allowed free choice of arms, but once an arm was entered, the divider prevented access to the other. To mitigate side bias, the divider blocked access to the arm entered by the rat in the previous trial on the fifth and tenth trials. Rats advanced to the barrier training if they selected the high reward on six of the eight free-choice trials on the last day of discrimination training.

Barrier training

Prior to barrier training, rats were acclimated to triangular barriers (15 cm and 30 cm) in home cages for 15 min. The first training phase introduced a small metal grid barrier (15 cm) in the high-reward arm. The initial day consisted of ten trials, while the subsequent days

consisted of eight. The first five trials of day one allowed free exploration and barrier climbing. Successful trial completion required consuming rewards in both arms within 300 s. For the remaining trials, successful completion required entering and consuming the reward in one arm within 150 s (first-choice rule applied). In the second phase (eight daily trials per four days), the taller metal grid barrier (30 cm) was used to obstruct access to the barrier arm, and rats were given free choice between goal arms according to the first-choice rule. Progression to the next stage required completing at least 14 out of 16 trials during the final two days of barrier training.

Treatment

Rats were randomly assigned to the acute (n=15) or subchronic (n=16) treatment group. In the acute treatment group, rats were randomly allocated to receive two doses of either 8-OH-DPAT (8-hydroxy-DPAT hydrobromide; 0.25 mg/kg) (Tocris) or saline (B. Braun) in total: one dose administered 24 h and one 5 min before testing. Rats in the subchronic group received jelly cubes containing PCPA (4-chloro-DL-phenylalanine methyl ester hydrochloride; 150 mg/kg) (Sigma-Aldrich) or vehicle jelly cubes for five consecutive mornings. This regimen was chosen based on PCPA's low solubility and our preliminary data demonstrating a significant reduction in 5-HIAA levels following five days of oral PCPA treatment. Jelly cubes (2×2×2 cm) were prepared daily using distilled water, powdered milk, sugar, gelatin from porcin skin (Sigma-Aldrich), and 0.5 % carboxymethylcellulose solution (Sigma-Aldrich).

Testing phase

The testing phase comprised seven trials conducted five days after barrier training. Six of seven trials involved a 30 cm barrier obstructing the reward in the barrier arm (*effort trials*). In the remaining trial (*no-barrier control trial*), conducted as the fifth in the sequence, the barrier was removed while maintaining reward placement. This trial assessed reward preference without the effort requirement.

Sample processing and preparation

Immediately after the rats completed their testing sessions, they were quickly anesthetized by inhalation of isoflurane vapors (Noviko) and decapitated. The brains were removed and dissected on ice. The tubes containing the PFC, striatum, and hippocampus were homogenized (UP50H, Hielscher Ultrasonics) in pure methanol (dilution coefficient 1:4) (Sigma-Aldrich). Next, samples were centrifuged (20000× g; Hermle Labortechnik) for 15 min at 4 °C. The supernatants were transferred into micro-inserts (Supelco), inserted in glass vials, and stored at -80 °C until processed.

High-performance liquid chromatography-tandem mass spectrometry analysis

Chromatographic separation was performed using a Thermo Ultimate 3000 system (Thermo Fisher Scientific) coupled with a QTrap 6500+ mass spectrometer (AB Sciex) equipped with Kinetex Polar C18 Column (2.6 µm; 150×3.0 mm; Phenomenex). The mobile phase consisted of two components: A, comprising 0.1 % formic acid (Sigma-Aldrich) in water, and B, composed of 0.1 % formic acid in pure methanol (Sigma-Aldrich). The flow rate was set to 250 µl/min, and the temperature was maintained at 40 °C. The elution profile followed a linear gradient from 5 % B (0-0.5 min) to 98 % B (4-5 min), with the initial gradient conditions restored within 30 s (5-5.5 min). The last minute of the HPLC method (5.5-6.5 min) maintained the starting conditions. Both HVA and 5-HIAA were analyzed in negative electrospray ionization (ESI-) mode. The ESI source parameters were set as follows: ion source gas one at 50 psi, ion source gas two at 45 psi, and curtain gas at 40 psi. The ion spray voltage was maintained at 5,500 V for ESI+ and -4,500 V for ESI-. The source temperature was held constant at 400 °C for both ionization modes.

Data analysis, visualization, and statistical analysis

Behavioral outcomes included goal arm choice (entry with more than half the rat's body) and goal arm latency (time from trial onset to goal arm entry). Goal arm choice was recorded during the experiment, while a blinded experimenter assessed goal arm latency from video recordings using Boris software. Statistical analyses were conducted using IBM SPSS Statistics and Estimation Stats [31]. Data were presented using GraphPad Prism, Estimation Stats, and Corel Draw. The effects of 8-OH-DPAT and PCPA treatment were compared only to their respective controls due to differences in treatment length and route of administration.

Results

Behavioral observations

During the last training trials (second phase of

barrier training), all included naïve rats reliably chose the barrier arm (average barrier arm selection rate=98.5 %) with no differences in goal arm latency (rmANOVA; $F_{(3,26)}$=0.269, p=0.847). Altogether, 31 out of 32 rats advanced to the testing phase; one rat was excluded as it failed to select the high reward on six of the eight free-choice trials on the last day of discrimination training. Rats that advanced to the testing phase were split into the acute group (acute saline (n=7) or 8-OH-DPAT (n=8) treatment) and the chronic group (vehicle jelly (n=8) or PCPA-containing jelly (n=8)).

During effort trials, 8-OH-DPAT treatment significantly decreased barrier arm selection (Fisher's exact test; *p(two-tailed)*<0.005) (Fig. 2A). Although the 8-OH-DPAT group exhibited increased barrier arm choices on the final trial compared to the first, this difference was not significant (McNemar's test; *p(two-tailed)*=0.375) (Fig. 2B). In contrast, PCPA treatment did not influence barrier arm choices during effort trials (*p(two-tailed)*=0.243) (Fig. 2C). Neither 8-OH-DPAT nor PCPA treatments affected high reward arm choices in the no-barrier control trial (Fisher's exact test; *p(two-tailed)*=0.473 and *p(two-tailed)*=0.467, respectively).

The 8-OH-DPAT treatment significantly increased goal-arm latency during effort trials (rmANOVA; $F_{(1,12)}$=10.708, p=0.007) (Fig. 2D). The goal-arm latency decreased throughout the sessions (rmANOVA; $F_{(2.376,28.507)}$=4.484, p=0.016); however, the interaction between trial order and treatment was not significant (rmANOVA; $F_{(2.376,28.507)}$=1.870, p=0.167), indicating that the effect of trial order on performance did not differ between the control and 8-OH-DPAT group. PCPA did not affect goal-arm latency during effort trials (rmANOVA; $F_{(1,14)}$=0.096, p=0.763) (Fig. 2E). The main effect of trial order, which reflects changes in performance across trial order regardless of treatment, was significant (rmANOVA; $F_{(2.533,35.460)}$=4.260, p=0.015), but the interaction between trial order and treatment was not significant (rmANOVA; $F_{(2.533,35.460)}$=1.870, p=0.258). Neither 8-OH-DPAT (2.03±0.24 s) nor PCPA (1.89±0.46) treatments affected the goal-arm latency (permutation t-test; p=0.474 and p=0.675, respectively) during no-barrier control trial.

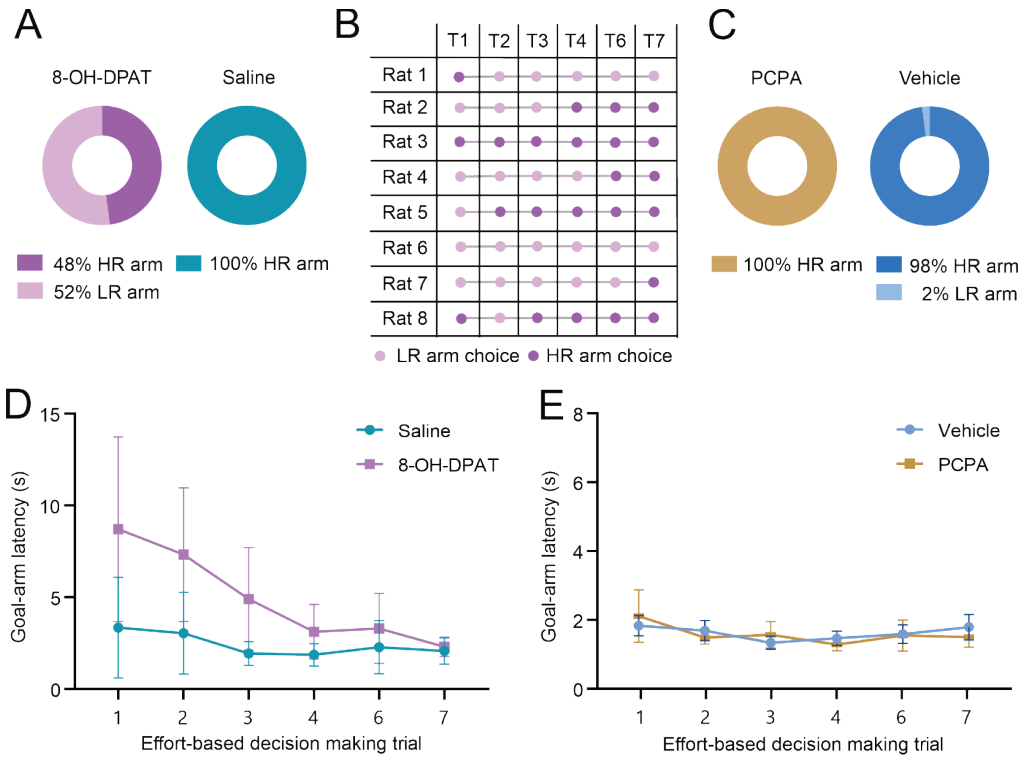

Fig. 2. The behavior of rats during effort trials. (**A**) The overall percentage of barrier and no-barrier arm choices by rats in the acute treatment groups. 8-OH-DPAT treatment significantly reduced the barrier arm selection rate. (**B**) Overview of goal-arm choices for each rat treated with 8-OH-DPAT during individual trials. (**C**) The overall percentage of barrier and no-barrier arm choices by rats in the chronic treatment groups. PCPA treatment did not affect the barrier arm selection rate. (**D**) The goal-arm latency of rats acutely treated with 8-OH-DPAT vs. saline and (**E**) rats that received subchronic PCPA treatment compared to vehicle only. 8-OH-DPAT treatment significantly increased goal-arm latency, while PCPA treatment had no effect. Results are reported as AVG ± SEM.

Prefrontal cortex

The acute 8-OH-DPAT treatment significantly reduced 5-HIAA concentration (permutation *t*-test; p=0.0444; Hedge's g=-1.08 [95.0%CI -2.13, 0.154]) (Fig. 3A), but did not affect HVA concentration in PFC (permutation *t*-test; p=0.855; Hedge's g=-0.104 [95.0%CI -1.25, 1.24]) (Fig. 3B). Subchronic PCPA treatment significantly decreased 5-HIAA concentration in the PFC (permutation *t*-test; p<0.01; Hedge's g=-3.23 [95.0%CI -4.67, -1.93] (Fig. 3C). Similarly to 8-OH-DPAT treatment, PCPA treatment did not affect HVA concentration in the PFC (permutation *t*-test; p=0.554; Hedge's g=0.331 [95.0%CI -0.802, 1.32]) (Fig. 3D).

Striatum

The 8-OH-DPAT treatment had no significant effect on either 5-HIAA (permutation *t*-test; p=0.674; Hedge's g=-0.264 [95.0%CI -1.51, 0.973]) or HVA levels (permutation *t*-test; p=0.527; Hedge's g=0.333 [95.0%CI -1.01, 1.65]) in the striatum (Fig. 4A-B). On the other hand, subchronic treatment with PCPA significantly reduced 5-HIAA concentration in the striatum (permutation *t*-test; p<0.001; Hedge's g=-6.33 [95.0%CI -7.76, -4.66]) (Fig. 4C). Similarly to 8-OH-DPAT treatment, PCPA treatment did not significantly affect striatal HVA levels (permutation *t*-test; p=0.641; Hedge's g=0.243 [95.0%CI -0.972, 1.19]) (Fig. 4D).

Hippocampus

The acute 8-OH-DPAT treatment significantly reduced both 5-HIAA (permutation *t*-test; p=0.0342; Hedge's g=-1.04 [95.0%CI -1.81, 0.942]) and HVA concentration in the hippocampus (permutation *t*-test; p=0.0036; Hedge's g=-1.88 [95.0%CI -2.85, -0.886]) (Fig. 5A-B). The subchronic PCPA treatment significantly decreased 5-HIAA concentration in the hippocampus (permutation *t*-test; p=0.0002; Hedge's g=-3.3 [95.0%CI -5.11, -1.73]) (Fig. 5C), but did not affect HVA concentration (permutation *t*-test; p=0.758; Hedge's g=-0.179 [95.0%CI -1.71, 0.941]) (Fig. 5D).

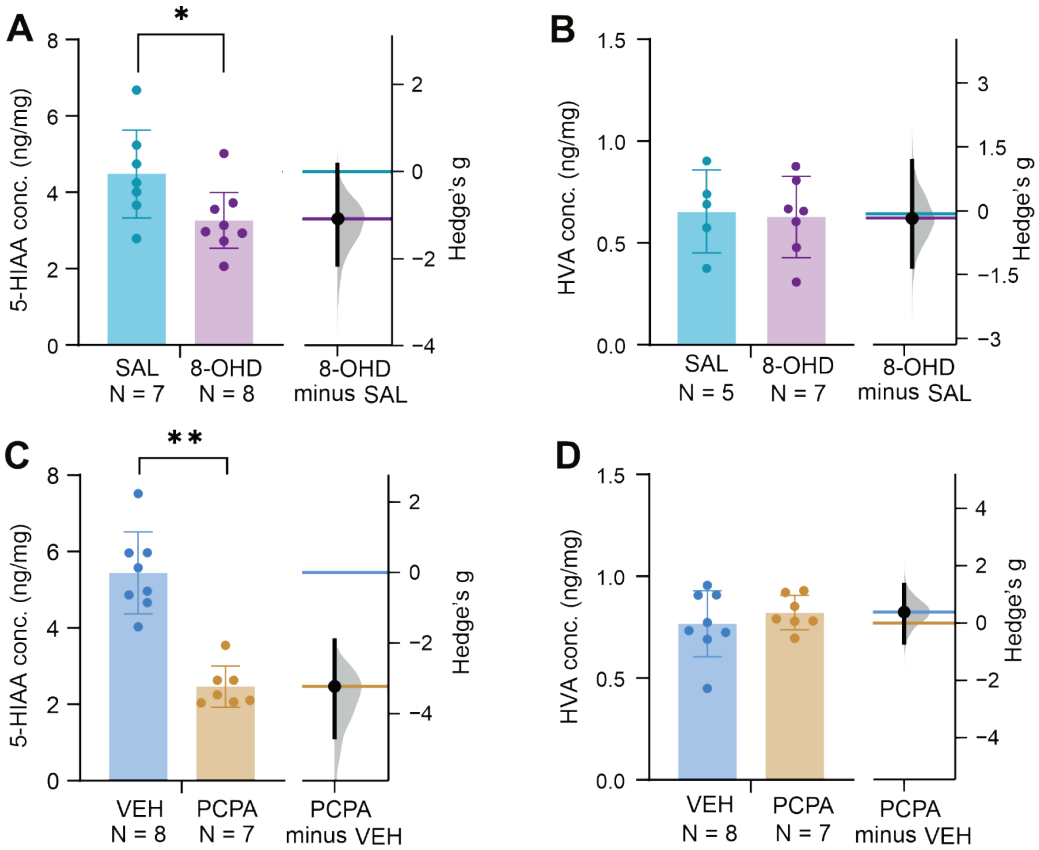

Fig. 3. The concentration of 5-HIAA and HVA in the PFC. (**A**) The acute treatment with 8-OH-DPAT (0.25 mg/kg) significantly decreased 5-HIAA levels compared to saline (3.264±0.312 ng/mg and 4.481±0.467 ng/mg, respectively), but (**B**) did not affect HVA concentration in PFC (0.625±0.084 ng/mg vs. 0.647±0.070 ng/mg in the saline controls). Similarly, (**C**) subchronic oral PCPA treatment (150 mg/kg) significantly reduced 5-HIAA levels (2.464±0.213 ng/mg compared to 5.567±0.345 ng/mg in the vehicle group), but (**D**) did not alter HVA levels (0.821±0.030 ng/mg compared to 0.767±0.053 ng/mg in the vehicle group). Results are reported as AVG ± SEM. SAL refers to saline control, 8-OHD refers to 8-OH-DPAT, and VEH corresponds to vehicle control. The statistical significance is denoted with asterisks, where * represents p<0.05, and ** indicates p<0.01.

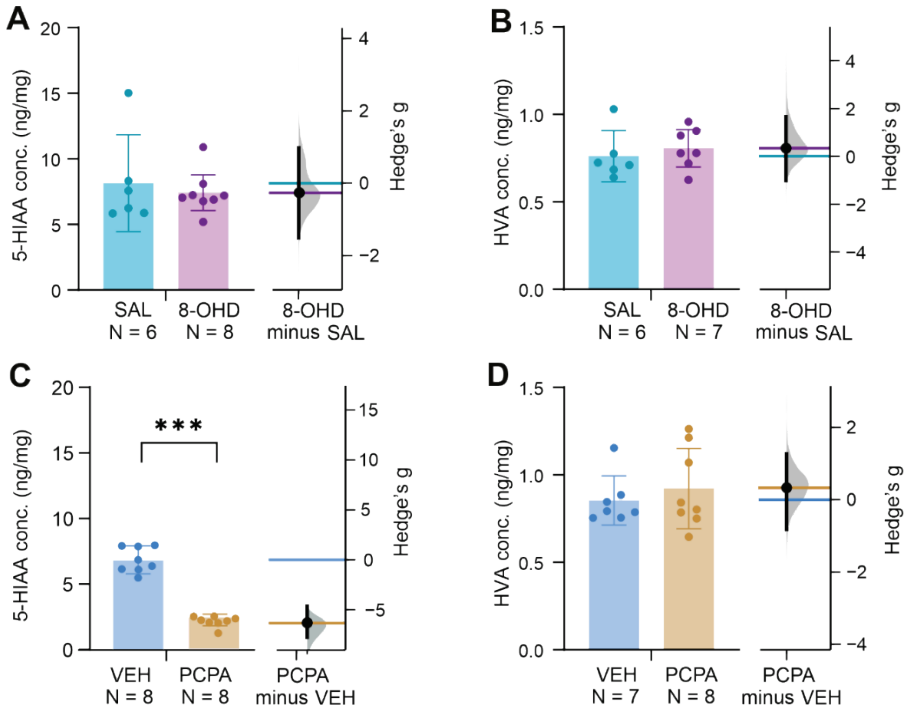

Fig. 4. The concentration of 5-HIAA and HVA in the striatum. The acute subcutaneous injection of 8-OH-DPAT (0.25 mg/kg) resulted in no statistically significant change in either (**A**) 5-HIAA levels (8.146±1.316 ng/mg and 7.417±0.545 ng/mg, respectively) or (**B**) HVA levels compared to saline (0.761±0.051 ng/mg and 0.806±0.040 ng/mg, respectively). (**C**) The subchronic oral PCPA treatment (150 mg/kg) significantly reduced 5-HIAA levels (2.041±0.120 ng/mg vs. 6.955±0.299 ng/mg in the vehicle group), but (**D**) did not affect HVA levels (0.922±0.078 ng/mg compared to 0.839±0.045 ng/mg in the vehicle group). Results are reported as AVG ± SEM. Results are reported as AVG ± SEM. SAL refers to saline control, 8-OHD refers to 8-OH-DPAT, and VEH corresponds to vehicle control. The statistical significance is denoted with asterisks, where *** indicates $p<0.001$.

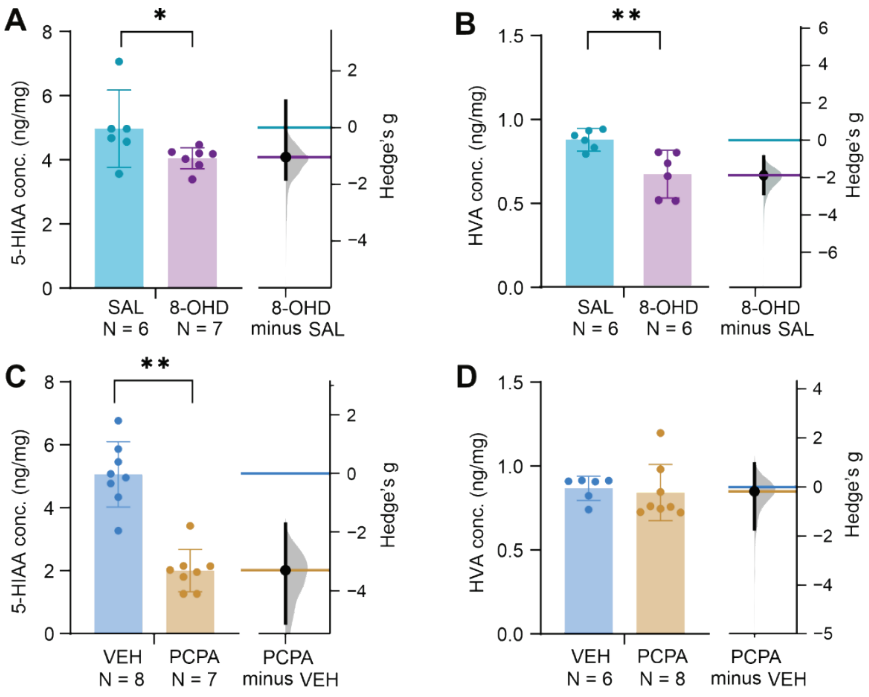

Fig. 5. The concentration of 5-HIAA and HVA in the hippocampus. (**A**) Acute 8-OH-DPAT injection (0.25 mg/kg) significantly reduced both 5-HIAA levels (4.050±0.127 ng/mg vs. 4.967±0.442 ng/mg in saline controls) and (**B**) HVA levels in the hippocampus (0.644±0.049 ng/mg vs. 0.845±0.021 ng/mg in saline controls). (**C**) Subchronic oral PCPA treatment (150 mg/kg) substantially reduced 5-HIAA levels (2.003±0.227 ng/mg vs. 5.062±0.377 ng/mg in the vehicle group) (**D**) but did not affect HVA levels in the hippocampus (0.842±0.050 mg/mg vs. 0.868±0.025 ng/mg in the vehicle group). Results are reported as AVG ± SEM. SAL refers to saline control, 8-OHD refers to 8-OH-DPAT, and VEH corresponds to vehicle control. The statistical significance is denoted with asterisks, where * represents $p<0.05$, and ** indicates $p<0.01$.

Discussion

The present study investigated the impact of sub-chronic oral PCPA and acute administration of 8-OH-DPAT on effort-based decision-making in rats. Our findings help delineate serotonin's role in decision-making, with distinct outcomes observed under PCPA and 8-OH-DPAT treatment.

We found that sub-chronic PCPA treatment did not affect the preference for the barrier arm, suggesting no impact of reduced serotonin synthesis on effort-based choices. These results align with previous research [32,33], demonstrating that decreased serotonin synthesis does not diminish rats' willingness to exert effort for a larger reward. However, while prior studies reported increased impulsivity (e.g., in delay discounting), our study did not observe differences in goal-arm latency.

In contrast, acute 8-OH-DPAT administration increased decision-making time and a preference for the less effortful option, as evidenced by reduced barrier arm entries and increased goal arm latency during effort trials. Moreover, during no-barrier control trials, 8-OH-DPAT-treated rats exhibited no differences in high-reward preference or goal arm latency compared to controls. While we did not specifically assess motor skills following 8-OH-DPAT treatment, several reports suggest that the selected dose (0.25 mg/kg) should not cause gross motor deficits. For example, in a model of traumatic brain injury, sham-operated controls treated with 8-OH-DPAT (0.5 mg/kg) did not exhibit deficits in motor function as assessed using balance and beam-walk tasks [34]. In the Morris water maze task, 8-OH-DPAT-treated rats (0.25 and 0.5 mg/kg) could swim without difficulty, although their swim times were longer [35]. 8-OH-DPAT treatment also caused no differences in treadmill running activity at doses 0.2 mg/kg and 0.4 mg/kg [36]. In our observations, no gross motor deficits (e.g., falls, limping) were evident, and animals successfully climbed the barrier whenever they attempted. Thus, while 8-OH-DPAT may cause some motor alterations, these alone are unlikely to account for the observed performance differences fully. These results indicate that 8-OH-DPAT modulates effort-based decision-making in rats without affecting intrinsic preference for a high reward when the effort is minimal.

Several mechanisms may underlie the influence of 8-OH-DPAT on rats' effort-based decision-making, including reduced serotonergic tone, its impact on dopaminergic transmission, and 5-HT1A receptor agonism. Activation of 5-HT1A somatodendritic autoreceptors in the raphe nuclei may lower extracellular serotonin levels in projection areas [37,38]. However, as both PCPA and 8-OH-DPAT groups exhibited decreased 5-HIAA levels without parallel behavioral changes in the PCPA group, reduced serotonergic transmission alone cannot fully explain the observed effects.

Despite dopamine's established role as a key regulator of effort-based decision-making (reviewed in [39]), dopaminergic signaling changes do not appear responsible for the decision-making deficits observed in our experiment. Indeed, 8-OH-DPAT can mimic certain dopamine actions as a partial D2 agonist [40,41], and acute treatment with 8-OH-DPAT has been shown to increase the firing activity of dopaminergic neurons in the ventral tegmental area [42], potentially enhancing dopamine release in projection areas. However, behavioral studies have reported decision-making deficits under dopamine blockade or depletion (reviewed in [43]). Contrastingly, an increase in dopamine neuron firing rate, which 8-OH-DPAT induces, improved decision-making by promoting the selection of high-effort, high-reward options [44]. The dopamine signaling changes associated with decision-making deficits are also typically related to the ventral striatum [1], which we did not observe in our experiment.

8-OH-DPAT may activate postsynaptic 5-HT1A heteroreceptors, which are especially abundant in the hippocampus and prefrontal cortex [45,46], subsequently modulating GABA and glutamate release [47,48]. 5-HT1A receptors are implicated in various forms of decision-making, including delay discounting, attentional set-shifting, reversal learning, and response inhibition (reviewed in [49]). In line with the high density of 5-HT1A receptors in the hippocampus, these receptors also play a prominent role in cognition and memory (reviewed in [50]), which may contribute to or directly cause impaired decision-making. Concomitantly, 8-OH-DPAT treatment was shown to cause learning and memory deficits [51,52]. Under the influence of the drug, rats may select the less rewarding option due to misremembering or failing to recall the association between barrier climbing and the high reward, as the reward is not visible from the cross-section.

The effect on 5-HT1A receptors may be one of the key differences in PCPA and 8-OH-DPAT action − while PCPA reduces the likelihood of postsynaptic 5-HT1A receptor activation due to decreased serotonin availability, 8-OH-DPAT acts as a direct 5-HT1A

receptor agonist. This hypothesis aligns with the findings that fluoxetine, a serotonin transporter inhibitor that elevates serotonin availability at the synaptic cleft, suppressed rats' preference for high-effort activities in a different effort-based decision-making task [53]. However, other factors, such as synergistic effects [54] or functional interaction between 5-HT1A receptors and other receptors [55], may also influence the overall effect, necessitating a more in-depth investigation.

Our study possesses two limitations. First, we only assessed serotonergic and dopaminergic transmission indirectly through their metabolites 5-HIAA and HVA concentrations. We selected this approach because 8-OH-DPAT has a known duration of action of approximately two hours in rats [56], and the decreased 5-HIAA levels should reflect the inhibition of serotonin release or synthesis [38,57]. As the sample collection occurred approximately 90±10 min after the injection, we estimated that serotonin/dopamine level assessment at the time of sample collection may not reliably reflect the peak effect of 8-OH-DPAT treatment. Second, we did not include female subjects in this study due to the lengthy training required, which did not allow us to acquire a sufficient sample size to statistically account for hormonal changes during the estrous cycle. Moreover, estradiol, a key hormone of the estrous cycle, has influenced effort-based decision-making [58].

Our results highlight the differential effects of pharmacological modulation of the serotonergic system on effort-based decision-making in male rats by 8-OH-DPAT and PCPA. Acute 8-OH-DPAT treatment altered decision-making and was associated with a 5-HIAA concentration decrease in the PFC and hippocampus and HVA levels in the hippocampus. Sub-chronic PCPA treatment had greatly decreased 5-HIAA levels in all observed regions but did not affect effort-based decision-making. We concur that the overall reduction of serotonin levels alone does not affect effort-based decision-making. However, our study highlights the possible role of the hippocampus and the 5-HT1A receptor in effort-based decision-making. Further research is warranted to elucidate the underlying mechanisms and extend the research to other brain regions of interest.

Conflict of Interest

There is no conflict of interest.

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