Distinct neuropharmacological substrates mediate impulsive action and impulsive choice in rats.

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Introduction

Impulsivity is currently thought to be a heterogeneous construct that is comprised of as two subcomponents: impulsive action and impulsive choice (see Winstanley et al., 2006 for a review of animal models of impulsivity). There is increasing evidence for dissociable neurobiological substrates underlying these constructs.

Premature responding in the 5-choice serial reaction time task (5-CSRTT), which has been described in detail elsewhere (e.g., Bari et al., 2008), is hypothesized to provide a measure of impulsive action, i.e., a failure to withhold an inappropriate response. The 5-CSRTT is a pre-clinical analogue of the continuous performance test in humans.

The delay discounting task (DDT) is a cross-species task used to assess impulsive choice, defined as intolerance of reward delay (Winstanley et al., 2006), where high impulsivity detected in the task is thought to be a trait of attention deficit-hyperactivity disorder (ADHD) patients and other patient populations. In animals, the DDT has been used to assess preclinical efficacy of ADHD medications, and additional explorations of neuropharmacological substrates of impulsivity.

Atomoxetine (Strattera®, 'ATX'), a norepinephrine transporter (NET) inhibitor, attenuates impulsivity in preclinical studies (for review, see Eagle and Baunez, 2010) and is an efficacious ADHD medication (e.g., Chamberlain et al., 2007).

The mixed dopamine/norepinephrine (DA/NE) reuptake inhibitor methylphenidate (Ritalin®) increases NE and DA overflow in the PFC and striatum, unlike atomoxetine which has no striatal effects (Bymaster et al., 2002), and is an effective ADHD medication (e.g., Shiels et al., 2009).

Multiple studies have implicated the $5HT_{2C}$ and $5HT_{2A}$ receptors in behavioral measures of impulsivity. For example, the $5HT_{2A/C}$ antagonist ketanserin decreased impulsivity in the 5-CSRTT but not the DDT (e.g., Talpos et al., 2006). $5HT_{2A}$ blockade attenuated impulsivity (e.g., Higgins et al., 2003), whereas $5HT_{2C}$ blockade increased impulsivity, in the 5-CSRTT (e.g., Winstanley et al., 2004). Most relevant to the present study, $5HT_{2A}$ blockade attenuated high levels of premature responding observed under prolonged ITI (9 s) conditions in the 5-CSRTT (Fletcher et al., 2011).

The present studies assessed the effects of atomoxetine, methylphenidate, ketanserin and SB242084 in the 5-CSRTT, hypothesized to provide a measure of impulsive action, and the DDT, hypothesized to provide a measure of impulsive choice. Based on the observed dissociable effects of these ligands, follow-up studies determined whether co-administration of 5HT receptor subtype ligands and NE/DA reuptake inhibitors yielded additive effects on therapeutic efficacy.

Methods:

Animals

Male Long-Evans rats (275-300g) were obtained from Harlan Laboratories (Indianapolis, IN). Upon arrival, the rats were assigned unique identification numbers (tail marked). Rats were single-housed in standard or OptiRAT cages and acclimated for 7 days prior to commencing a food-restriction regimen: rats were held at 85% of age-matched free-feeding control body-weights, receiving approximately 10-20 g of rat chow daily. Water was provided ad libitum, except during testing. Animals were maintained in a 12/12 h light/dark cycle (lights on at 0700 EST) with room temperature maintained at 22 ± 2°C and the relative humidity maintained at approximately 50%. All animals were examined, handled and weighed prior to initiation of the study (during the week of habitation) to assure adequate health and suitability and to minimize non-specific stress associated with testing. All efforts were made to minimize discomfort of any sort at all times during the conduct of the studies. Behavioral test sessions were performed during the animal's light cycle phase. All experiments were approved by the Institutional Animal Care and Use Committee of PsychoGenics, Inc in AAALAC-accredited facilities, and in accordance with the Guide to the Care and Use of Laboratory Animals (NIH, 2010).

Apparatus

The test apparatus consisted of 10 aluminum and Plexiglas chambers with grid floors (width 31.5 cm, depth 25.0 cm, height 33.0 cm), housed in sound-attenuating cabinets (Med Associates, St. Albans, VT). Each cabinet is fitted with a low-level noise extractor fan. The left wall of each chamber was concavely curved with 5 apertures evenly spaced, located approximately 2.5 cm from the floor. Each aperture contained a standard 3W LED to serve as stimulus lights. The opposite wall contained a food magazine, located approximately 3.0 cm from the floor. A retractable lever (approximately 2 cm long) was positioned on either side of the food magazine (approximately 2 cm from the grid floor). Each chamber was illuminated with a 1.5W house-light located above the food magazine, 1 cm from the chamber ceiling. After each test session the apparatus was cleaned with 70% ethanol; at least 20 minutes elapsed before the next test session. The test session functions were controlled, and all data were captured, by a computer using K-Limbic software (Conclusive Software Ltd., distributed by Med Associates, St. Albans, VT).

Drugs

Methylphenidate hydrochloride was obtained from Sigma-Aldrich, Missouri, USA. Tomoxetine hydrochloride, ketanserin and SB242084 were obtained from Tocris Biosciences, Missouri, USA. Test compounds were dissolved in sterile saline and administered IP in a volume of 1 ml/kg (doses expressed as salt form). Atomoxetine, methylphenidate, ketanserin and SB242084 were administered with pre-treatment times of 30 minutes. In the co-administration studies (Experiment 5), test compounds were administered immediately after each other in two discrete injections. In all studies, all rats received all drug treatments, according to a randomized-order, counter-balanced and within-subjects design ('Latin square' design). The 5-CSRTT studies were performed in 9-11 rats and the DDT studies were performed in 10-11 rats (group sized specified in Figures). In all cases, animals only entered a study if they exhibited stable baseline performance that met the acquisition criteria specified above. For the 5-CSRTT studies, rats were used for 1-3 experiments. For the DDT studies, rats were used in 2-5 experiments. Drug tests were performed on Wednesdays and Fridays of each week, when subjects met pre-determined performance criteria.

Statistical Analysis

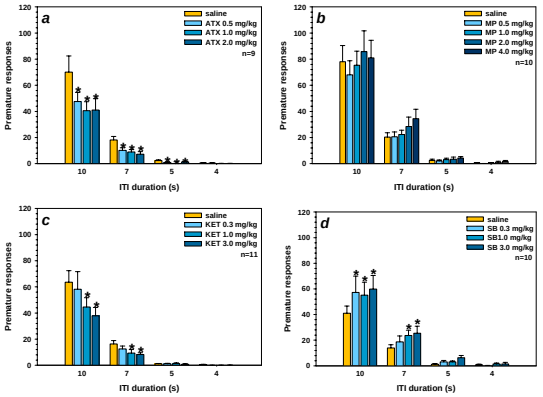
Data were analyzed via one- (Study defined as a within-subjects factor) or two-way ANOVAs (ITI/Delay duration and Dose defined as within-subjects factors). Where the assumption of equal variance was violated, data were transformed to the square root prior to ANOVA. ANOVAs were followed, when appropriate, by Student Newman-Keuls post-hoc tests.

Study 1: Atomoxetine and Ketanserin, but not methylphenidate or SB242084, attenuated impulsive action, as measured in the 5-CSRTT.

Procedure

Animals were trained to monitor the five apertures for stimulus light illumination: a nosepoke into the illuminated aperture resulted in delivery of a food pellet. Nose-pokes made during the inter-trial interval are defined as premature responses and are punished by the imposition of a time-out (5 s); premature responses resulted in a reset of the ITI. Rats took 40-60 sessions to acquire the task. Variation of the inter-trial interval (10, 7, 5 or 4 s) in rats trained in a 5-s ITI task resulted in increased premature responding at longer ITI's.

Other measures obtained during the test sessions were: (1) percent correct (number of correct trials X 100, divided by the total number of correct and incorrect trials, (2) percent omissions (number of missed trials X 100 divided by total number of trials), (3) perseverative responding (additional responses emitted after the initial nose-poke within a single trial,) (4) correct and incorrect response latencies, (time to make a correct /incorrect response after the illumination of the stimulus, (5) magazine latency (time taken to enter the food magazine after a correct response.



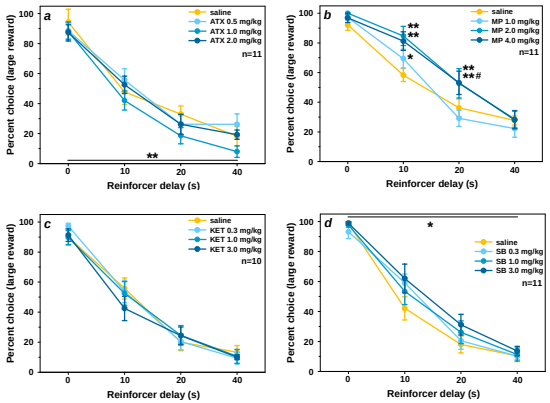
Results: Atomoxetine and ketanserin significantly decreased premature responses at 10, 7 and 5 s (ATX only) ITI trials (*p<0.05; Panels ***a***, ***c***). Methylphenidate had no significant effects on premature responding (Panel ***b***). SB242084 significantly increased premature responses at 10 and 7 s ITI trials (*p<0.05; Panel ***d***). Data are expressed as mean ± SEM. There were minimal effects of test compounds on other test measures (data not shown).

Study 2: Methylphenidate or SB242084 but not Atomoxetine or Ketanserin, attenuated impulsive choice, as measured in the DDT.

Procedure

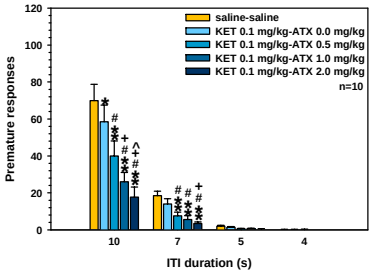
Animals were trained to respond on one lever for a large (3 pellets) reward delivered at variable delays (0, 10, 20 and 40 s) after pressing the lever, and to respond on a different lever for a small (1 pellet) reward delivered immediately after the lever-press. Trials were presented in four distinct blocks of 12 trials: the first four trials of each block were forced trials (i.e., only one lever was presented; each lever was presented twice), and the final eight trials were free choice trials (i.e., both levers were presented). The designation of left/right lever as delayed/immediate reward was counter-balanced across subjects. Percent preference for the large reinforcer as a function of delay: calculated as the number of choices for the large reinforcer/(total number of choices for large + small reinforcers) × 100; calculated for each specific delay duration;

Additional measures obtained were (1) percent omissions: failures to respond when magazine is illuminated or lever is presented: calculated as the number of omissions/(total number of correct responses + omissions) X 100 (forced and choice trials); (2) response latency: the time to lever-press from the extension of the levers (for choice trials only); (3) magazine latency: the time from food pellet delivery to collection of the food pellet from magazine (for all trials).



Results: Administration of atomoxetine (Panel ***a***) decreased tolerance for reward delay (significant main effect of Dose, *p<0.05). By contrast, administration of methylphenidate (Panel ***b***) compared to vehicle (*p<0.05, **p<0.01 pound signs), increased tolerance for reward delay at the two intermediate delays (10 and 20 s). In addition, 4.0 mg/kg methylphenidate significantly increased reward delay tolerance compared to 1.0 mg/kg methylphenidate at 20 s delay. Although administration of the $5HT_{2A/C}$ antagonist ketanserin (Panel ***c***) was ineffective, the $5HT_{2C}$ antagonist SB242084 (Panel ***d***) increased reward delay tolerance (significant main effect of Dose, *p<0.05). Data are expressed as mean ± SEM.

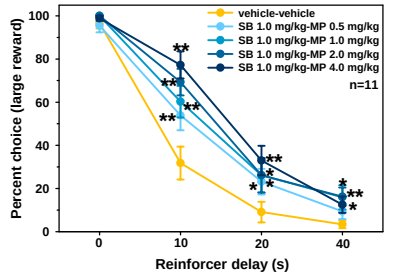
Study 3: Co-administration of atomoxetine and ketanserin attenuated impulsive action in the 5-CSRTT.



Co-administration of ketanserin and atomoxetine tended to enhance the effects of atomoxetine alone. Premature responding data are expressed as mean ± SEM. Asterisks (*p<0.05, **p<0.01) indicate significant differences compared to vehicle-vehicle at specific inter-trial interval values. Pound, plus and carat signs indicate significant differences compared to co-administration of ketanserin and 0.0 (#p<0.05), 0.5 (+p<0.05) or 1.0 (^p<0.05) mg/kg atomoxetine at specific inter-trial interval values.

Study 4: Co-administration of methylphenidate and SB242084 attenuated impulsive choice in the DDT.

Co-administration of methylphenidate and SB242084 enhanced reward delay tolerance in the DDT. Percent choice of large reward data are expressed as mean ± SEM. Asterisks (*p<0.05, **p<0.01) indicate significant differences compared to vehicle-vehicle at specific reward delay values.



Summary:

The norepinephrine reuptake inhibitor atomoxetine attenuated premature responding in the 5-CSRTT, but was ineffective in the DDT.

The mixed dopamine/norepinephrine reuptake inhibitor methylphenidate exhibited an opposite profile of effects.

Blockade of $5HT_{2A/C}$ receptors via ketanserin decreased premature responding but had no effects on percent choice for delayed reward; blockade of $5HT_{2C}$ receptors via SB242084 had opposite effects.

Follow-up studies provided some limited evidence of additive effects of $5HT_{2A/C}$ receptor blockade on the effects of atomoxetine on impulsive action.

These studies demonstrate dissociable profiles of stimulant versus non-stimulant ADHD medications, and 5HT subtype-selective ligands, in the 5-CSRTT and DDT assays. Thus, the present findings support the sub-categorization of impulsivity, and suggest that 5HT receptor subtype-selective antagonists may provide therapeutic targets for disorders characterized by different forms of impulsivity.

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