Research Paper

Long-term behavioural effects of the "Legal High" MDMA alternative 5-IAI in adolescent rats

David M Compton, Emily M, Cnapich BS, Ashley Fravel, Jordan M Sossong

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Previous research reports have suggested the emergence of novel club drugs as "legal high" alternatives to illegal hallucinogenic or entactogenic drugs such as 3,4-methylenedioxymethamphetamine (MDMA). Among these are recent reports of the use of psychoactive derivatives of 2-aminoindane, including 5-Iodo-2-aminoindan (5-IAI). While much is known about the effects of MDMA, little is known about the physiological and cognitive effects of 5-IAI. When considered in terms of exposure during the neuropsychological development period of adolescence, the available information is completely absent. In the present investigation, beginning at 35 days of age rats were given 20 mg/kg doses of 5-IAI or saline until the animals reached adulthood. Behavioral testing occurred in adulthood when the rats were 124 days old and had been drug free for 65 days. Our assessments included measures of general activity, stepdown passive avoidance, and a series of Morris water maze spatial and non-spatial memory tasks. Depending on task demands, the performance of 5-IAI-treated rats was inferior to that of the saline control animals. However, unlike the changes seen following MDMA exposure, no differences in serotonin and dopamine levels were found. The results are discussed in the context of the disruptive effects that 5-IAI may have on adolescent brain development and how such compounds may contribute to cognitive deficits and maladaptive behavior.

Key Words: *5-Iodo-2-aminoindan, 5-IAI, MDMA, spatial learning, water maze, development, memory*

Approximately 27 years ago, a psychoactive analog of p-iodoamphetamine, 5-Iodo-2-aminoindan (5-IAI), was synthesized and reported for medical research as an analog of 4-iodoamphetamine, a MDMA (ecstasy) like compound [1,2]. According to Nichols et al., 5-IAI mimicked MDMA but without the neurotoxicity associated with MDMA [2]. Nonetheless, the lack of reliable references suggests that, unlike MDMA, the drug was largely ignored among club drug users for a number of years.

More recently, however, individuals in the club drug community began to suggest so-called "legal high" alternatives to elicit compounds such as MDMA (ecstasy), with discussions about the properties and effects of 5-IAI [3-6] appearing about 20 years after the first appearance in the scientific literature [2]. Accordingly, reports concerning its abuse potential began to appear in scientific and law enforcement publications [7-12]. Collectively, there is little reliable information on aminoindanes as a group [10]. First-person anecdotes on the effects of 5-IAI remain on the internet, yet no empirical study on the effects of this compound in human subjects has been reported [9,10].

While reports on the neuropsychological effects of 5-IAI are lacking, a pharmacological profile of 5-IAI has emerged. Similar to that of MDMA, 5-IAI preferentially inhibits the 5-HT transporter (SERT) as well as the norepinephrine transporter (NET), with effects on the dopamine transporter (DAT) observed [12,13]. However, it has been reported that the potency of MDMA was double that observed in 5-IAI [12,13]. In addition, similar 5-IAI effects on human monoamine transporters have been described [14]. Noteworthy, 5-IAI binding to 5-HT receptor sites has been reported [12]. Hallucinations following 5-IAI ingestion have been described [9], relevant because 5-IAI binds to 5-HT2A receptors which have been associated with the experience of hallucinations [9,15,16]. Given reports that 5-IAI has lower toxicity [2] yet successfully substitutes for MDMA in drug discrimination tests [2], the appeal of this drug as a novel alternative to MDMA is possible [9].

Substantial neural modification takes place during the adolescent period of development [17]. Among the myriad of changes associated with this period of physiological development are the alterations to serotonin (5-HT) neural systems during adolescence [18,19]. For example, differential expression of 5-HT receptors with age has been described [20]. Considered collectively, given the substantial anatomical and functional transformations associated with adolescent development, exposure to drugs during this time point in the lifespan can be particularly problematic [21]. Indeed, when the effects of adolescent MDMA exposure have been explored, reports appeared suggesting that such exposure produced a long-term decrease in 5-HT levels, as determined by measures of metabolites, binding sites, as well as direct neurotransmitter levels [22-26]. Further, when compared with adolescent mice, adult mice show a lower sensitivity to the reinforcing effects associated with MDMA [27]. Given that adolescent drug use is commonly associated with higher levels of dependence in adulthood [28] and that younger adults are more likely than older adults to develop addictions [29], the timing of exposure to different drugs with abuse potential is a salient issue.

In rats, adolescence spans the period from weaning (the 21st postnatal day, PND) until the 60th PND, when the animal has reached adulthood [30]. Adolescence can be delineated further as comprised of three developmental periods consisting of prepubescence or early adolescence (PND 21-34), periadolescence or mid adolescence (PND 34-46), and that of late adolescence/early adulthood (PND 46-59). Using this framework as a model of rodent development allows for comparative evaluations and extrapolation to humans [31]. Thus, the consideration of different adolescent age groups provides a framework for the examination of the developmental consequences associated with drugs of abuse at different time points in physiological and cognitive neurobehavioral development.

OBJECTIVE

The research attention associated with consideration of 5-IAI has largely focused on biochemical metrics of toxicity. Unfortunately, little consideration to the possible long-term neurocognitive and behavioral consequences of its use have been reported, with no reports the effects of 5-IAI during adolescent brain development. Therefore, in the present investigation we attempted to address some of the gaps in the literature. Specifically, our study comprised an experiment with the following goals:

1. To investigate the long-term effects of 5-IAI following exposure during adolescence employing common behavioral models to test different aspects of learning and memory, and

Palm Beach Atlantic University, West Palm Beach, FL, United States

Correspondence: David M. Compton, Palm Beach Atlantic University, Department of Psychology, Gregory Hall, West Palm Beach , FL 3340.
E-mail: david_compton@pba.edu
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2. To test for any lingering changes on brain 5-HT and DA levels after adolescent exposure, followed by a period of abstinence.

SUBJECT AND METHODS

Subject

The subjects were of 16 male Long-Evans rats (Charles River, Wilmington, MA), 35 days of age at the beginning of drug exposure. All rats were individually housed in standard stainless-steel cages in a climate-controlled facility with an ambient temperature of 25°C with the humidity between 45% and 50%. The animals were maintained on a 12-h light/dark cycle with food (Mazuri Rodent Chow) and water provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Palm Beach Atlantic University and the animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals [32].

Drug exposure began when the rats were 35 days old, a period defined as mid-adolescence in rodent development. The 5-IAI drug dose was chosen based on the range (100 mg^{-1} gram) reported by recreational users [3-6] and half that of the 40 mg/kg reported by Nichols and colleagues (2). All rats received a total of 13 injections of 5-IAI (20 mg/kg; Cayman Chemicals, Ann Arbor, Michigan) or a corresponding injection volume of isotonic saline. Purity of the 5-IAI was verified by the suppliers using HPLC. During all drug exposure sessions, the ambient temperature was maintained at approximately 25°C with the humidity between 45% and 50%. Prior to the commencement of the experiment, the rats were randomly assigned to one of two groups and exposed to 5-IAI (n=8) or a comparable volume of saline (n=8) from PND 35 to PND 59 with injections spaced at 48-hour intervals. Behavioral testing occurred in adulthood when the rats were 124 days old and had been drug free for approximately 65 days.

Apparatus

Morris Water Maze (MWM)

With the exception of general activity and step-down passive avoidance testing, spatial and non-spatial assessment took place using a white circular plastic swimming pool (Morris water maze, MWM) with a diameter of 183cm. Extra-maze cues and escape parameters differed depending on the task requirements. For all assessment phases save one (see water maze tasks), water depth was held constant at 30 cm and made white in appearance using a nontoxic water-based paint (Sargant Art, Hazelton, PA). The MWM and extra-maze cues were located in a quiet 36.88 m^2 testing room. The use of white curtain panels surrounding the pool, obscured the available distal cues on two of four walls. With the except of probe trials, an escape platform (15 cm × 15 cm) and painted flat white was used throughout training and testing. The escape platform was located 18 cm from the wall of the swimming pool which forced the subjects to swim away from the swimming pool wall in order to find the platform. During the cued MWM phase, the escape platform protruded 15 mm above the surface of the water. The escape platform was submerged to a depth of 15 mm below the surface of the water for the remaining MWM phases of the experiment.

Procedure

Assessment of general activity and exploration

Rodent general levels of activity were measured across a two-day period with daily five-min assessment periods. General activity was measured in a 60.96 cm × 60.96 cm chamber consisting of black 10.16 cm squares with white edges. Here, the dependent measures included the number of squares crossed during the five-min period and the number of times the rats reared onto their hind legs. For the purposes of our experiment, rearing was defined as both forelimbs elevated, either freestanding or against a wall [33]. The activity of the animals was recorded and independently rated by two raters. Last, non-associative influences, such as general swimming ability and sensorimotor deficits were assessed using a cued version of the MWM task described below.

Step-down passive avoidance testing

A standard operant chamber (21-cm × 28-cm; Lafayette Model 84022) with a stainless steel electrified grid floor was used to assess passive avoidance conditioning. Located in the center of the floor, the chamber held a 10.14 cm × 10.14 cm platform. Whenever the rat left the platform and physically touched the grid floor, it experienced a 4mA foot shock.

MWM tasks

The MWM protocols (Figure 1) described here have been employed successfully to assess rodent learning and memory [34]. With the exception of the cued place learning phase, the platform was submerged to a depth of 15 mm below the surface of the water. For the cued place learning phase, the platform was 15 mm above the water's surface. On all trials the rat was gently released into the pool at one of four cardinal compass designations with a ceiling of 60 sec per trial to reach the escape platform. If the rat failed to reach the platform within the 60 sec period, it was gently placed there. After either successfully locating the platform or after 60 sec period had elapsed, the rats were allowed to rest on the platform for about 15 sec before the next trial. With the exception of probe trials, the escape platform was located in either the southwest, southeast, northeast, or northwest quadrants. Escape times to the escape platform were recorded with a stopwatch and errors, operationally defined as crossing one of four quadrants associated with the four cardinal compass points beyond the minimum required for the shortest distance from placement to escape, were recorded.

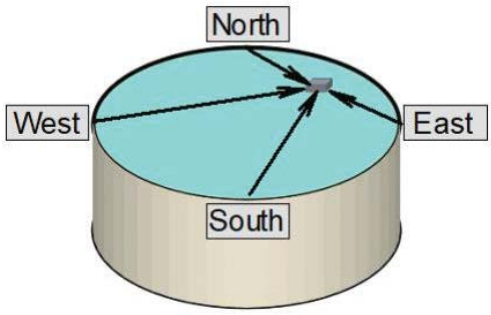

Figure 1) *The Morris water maze layout used in the present study. The platform is shown in grey but, like the water, is white in color in the actual maze. For the cued place learning phase, the platform was 15 mm above the water's surface. For the remaining phases, the platform was submerged to a depth of 15 mm. On all trials the rat was gently released into the pool at one of four cardinal compass designations. With the exception of probe trials, the escape platform was located in either the southwest, southeast, northeast, or northwest quadrants. The phase was followed by an easy place learning phase, with a number of extra-maze cues available. In a third phase, in order to increase the difficulty of the task, a curtain was be placed around the water maze and room lighted by a single 60-watt red light bulb, thus leaving minimal extra-maze cues to assist navigation. The last phase of testing, learning set acquisition, required the animals to learn a new escape platform location each day for five consecutive days.*

Simple (cued) place learning

The cued place learning MWM task began the day following step-down passive avoidance testing. Using a visible escape platform, we included this assessment phase to determine if non-associative influences, such as general swimming ability and non-declarative memory ability, could influence performance during the spatial place and learning set tasks. This testing phase included 10 trials per day for two consecutive days. The visible escape platform was located in one of four possible locations.

Spatial water maze tasks

Following cued place learning, two phases of the protocol with tests of spatial reference memory that varied in difficulty were considered. Here, the MWM tasks involved learning the location of a submerged platform that remained constant across all trials within a given phase of the experiment. However, in the standard (easy) version of the task when multiple extra-maze cues are available, quite often only minor deficits at most are typically seen [35], including in previous research in our lab. Therefore, two variations of the task were used. A latter, more difficult (hard) version of the place-learning task was also included, since it is considered more sensitive to spatial learning/memory impairments following adolescent drug exposure to MDMA [36]. As in the cued version, escape latency and number of quadrant errors were recorded.

Considered less cognitively demanding, a version of the MWM with a number of extra- maze cues lasted two days and consisted of training the rats for 10 trials per day. As before, the rats were permitted to rest on the platform for 15 sec at the completion of each trial. Further, a test of retention was conducted through the use of a probe trial on the second day of this phase. Here, the assessment consisted of a 60 sec "free swim" where the escape platform was removed not less than two hours after the last place learning trial. Time

spent swimming in the target quadrant and the number of crossings over the former platform location were recorded. The following day, the next place learning phase began and continued for five days consecutive days. A version of the MWM place-learning task, this version was considered more difficult since the availability of extra-maze cues to aid navigation was minimized. Specifically, a white curtain was placed completely around the water maze and in room lighting reduced to a single 60-watt red light bulb located beyond the curtain, below the horizon of the pool, and approximately three meters from the water maze. As a result, this effectively left the rat with few visual cues to aid navigation. The rats were trained four consecutive trials per day. On a given trial, after locating the escape platform, the rats were allowed to rest for 15 sec. Last, a daily probe trial was administered not less than two hours after the last trial of the daily four-trial series.

Spatial learning set

The last phase of testing, learning set acquisition, required the animals to learn a new location of the escape platform daily on each of five consecutive days. All animals received four consecutive trials per day. In order to find the escape platform quickly, this version of MWM requires the animal to recall its response on the immediately preceding trial. Therefore, comparison of the escape latencies on trial 1 versus trial 2 of each day was used as an index of working (short-term) memory. As before, the rats were allowed to rest on the platform for 15 sec at the completion of each trial.

Assessment of brain dopamine and serotonin levels

Twenty-one days following the end of behavioral testing the animals were euthanized and cortical and hippocampal DA and 5-HT levels were examined. The high performance liquid chromatography (HPLC; Waters Model 600 with electrochemical detection) procedures reported in the present experiment are based on a modified version described elsewhere [34,37]. Raw data were integrated and analyzed to determine 5-HT levels in two regions of the brain–the hippocampus, the prefrontal cortex. HPLC grade H_2O was used to create concentrations in the amounts of 0.04% sodium octyl sulfate, 0.1 mM disodiumethylenediamine-tetraacetate, 0.05 M sodium phosphate, with 0.03 M citric acid acting as a buffer. The aqueous portion of the mobile phase was maintained at pH levels between 2.7 and 2.9 and the mobile phase consisted of 20% methanol and 80% aqueous phase. The HPLC column was a Waters C18 reverse phase analytical column (3.9 unit × 300 mm; 4 μm). DA and 5-HT levels were calculated and analyzed as ng/g tissue.

Statistical analyses

Statistical analyses involved the use of mixed analysis of variance (ANOVAs), with drug group as the between-subjects factor and days or days and blocks of trials, as within-subjects factors. Post-hoc analyses were performed using TukeyHSD or paired t-tests with a Bonferroni correction to control for multiple comparisons. The alpha level for acceptance was set at $p<0.05$ and analyses were performed using SPSS [38].

RESULTS

General activity

Consideration of the activity data revealed the following. The number of squares traversed declined across days, $F(1,14)=4.89$, $p<0.05$, $\eta p2=0.259$, but the main effect of drug group and the group X days interaction were non-significant. Similarly, the number of rearing behaviors declined across days, $F(1,14)=4.78$, $p<0.05$, $\eta p2=0.255$. In addition, rearing behavior among 5-IAI rats (M=17.87, SD=8.28) was significantly higher than among control animals (M=8.63, SD=7.99), $F(1,14)=8.72$, $p<0.05$, $\eta p2=0.384$.

Stepdown passive avoidance

Analysis of the step-down avoidance data involved the use of a 2 (drug groups) X 2 (days) mixed ANOVA. Considerable heterogeneity in variance between the two groups was noted; therefore, the data were transformed using a reciprocal (X=1/X) transformation [39]. No differences in step-down latencies as a function of drug group were observed. A main effect of days was detected, $F(1,14)=12.89$, $p<0.01$, $\eta p2=0.489$, suggesting that the animals retained the memory of the adverse experience from the first day (Day 1:M=0.1178, SD=0.0514; Day 2:M=0.0515, SD=0.0568). Nevertheless, the drug group X day interaction was non-significant, indicating that group latencies were comparable across days.

Simple (cued) place learning

After initial consideration of the escape latencies, the data were transformed using the reciprocal transformation. The data associated with cued place learning were considered over the two days of testing with each day's data collapsed into blocks of five trials using a 1-between (drug), 2-within (days, trials) mixed ANOVA. The resulting analysis revealed significant improvements across days, $F(1,14)=18.93$, $p<0.01$, $\eta p2=0.575$, and blocks of trials, $F(1,14)=28.98$, $p<0.001$, $\eta p2=0.674$, as well as a days X blocks interaction, $F(1,14)=6.31$, $p<0.05$, $\eta p2=0.311$. Thus, escape latencies decreased as a function of experience both within trials and across the two-day test period. However, no drug associated effects were found.

Examination of the extra-quadrant crossings (i.e., errors) across the cued place-learning phase revealed a similar pattern of a significant main effects of days, $F(1,14)=7.64$, $p<0.05$, $\eta p2=0.353$, blocks of trials, $F(l,14)=37.41$, $p<0.001$, $\eta p2=0.728$, and a days X blocks interaction, $F(l,14)=7.34$, $p<0.05$, $\eta p2=0.344$. Therefore, all animals improved within blocks of trials as well as across days but no effects of drug were detected.

Easy place learning

Once again, examination of the escape latency suggested a need for a data transformation. After screening this set of escape latencies, the square root transformation (=√) was chosen [39]. As was the case with the cued place learning, the resulting data were analyzed using a 1-between (drug group), 2-within (2-days, 2 blocks of trials) mixed ANOVA. Although no effect of drug was found, the analysis indicated significant improvement across days, $F(1,14)=32.47$, $p<0.01$, $\eta p2=0.699$, and blocks of trials, $F(1,14)=45.25$, $p<0.001$, $\eta p2=0.764$, as well as a days X blocks interaction, $F(1,14)=6.96$, $p<0.05$, $\eta p2=0.332$. Thus, as with the cued place learning data, escape latencies decreased as a function of experience both across blocks of trials and across the two-day test period but no drug related impact was found. Similarly, examination of the errors across the simple place-learning phase revealed a similar pattern of a significant main effects of days, $F(1,14)=25.26$, $p<0.001$, $\eta p2=0.643$, blocks of trials, $F(l,14)=41.21$, $p<0.001$, $\eta p2=0.746$, and a days X blocks interaction, $F(l,14)=7.96$, $p<0.05$, $\eta p2=0.362$. Therefore, all animals improved across blocks of trials as well as across days. However, although no effect of group was found in when escape latencies were considered, for the number of extra quadrants cross (errors) a main effect of drug group was found, $F(l,14)=5.78$, $p<0.05$, $\eta p2=0.277$, with 5-IAI rats traversing more quadrants (M=5.13, SD=1.49) than the control rats (M=3.80, SD=0.58). Last, drug and saline-treated rats spent a comparable amount of time in the formerly correct escape quadrant while traversing a comparable number of quadrants.

Difficult place learning

This phase involved consideration of a place learning task with few allocentric cues available to facilitate navigation. Preliminary screening of the data suggested the need for the reciprocal data transformation. For each day of testing, the four daily trials were collapsed. Examination of the resulting data using a 1-Between (drug groups), 1-Within (days) ANOVA revealed a main effect of drug group, $F(1,14)=35.28$, $p<0.001$, $\eta p2=0.716$, suggesting differences in escape latencies as a function of drug condition that remained across the assessment period (Ms=0.066 vs. 0.121, 5-IAI & Saline rats, respectively). However, the main effect of days and the group X days interaction were non-significant.

Following data screening, the time spent in the target quadrant on probe trials was analyzed using the untransformed times. Consideration of possible spatial bias on probe trials revealed the following. Here, a main effects of days, $F(4,56)=14.95$, $p<0.001$, $\eta p2=0.516$, and drug group, $F(4,56)=7.92$, $p<0.05$, $\eta p2=0.361$, were found. With the exception of the 5th day, time spent in the formerly correct escape quadrant declined across days but the control animals (M=16.81sec, SD=2.13) spent significantly more time in this quadrant than the 5-IAI-treated rats (M==13.73sec, SD=2.04). However, a significant group X days interaction related to the time spent in the formerly correct escape quadrant, $F(4,56)=5.43$, $p<0.01$, $\eta p2=0.27.9$. Here, the group differences, while large on early test days, diminished across the 5-day period. Specifically, the greatest differences–and only significant group difference–were observed on day one of this phase of testing.

A similar pattern to the escape latency data was observed when the for the error data were examined. Here, a main effect of drug group was detected, $F(1,14)=19.09$, $p<0.01$, $\eta p2=0.577$, with 5-IAI rats traversing more quadrants (M=5.76, SD=1.58) than saline control rats (M=3.01, SD=0.81). In addition, the main effect of days was significant, $F(4,56)=3.71$, $p<0.01$, $\eta p2=0.210$, with quadrant errors declining across the testing period. On probe trials, main effects of days, $F(4,56)=4.70$, $p<0.01$, $\eta p2=0.251$, and drug group, $F(1,14)=4.86$, $p<0.05$, $\eta p2=0.258$, were found. Thus, the groups differed in

terms of the number of quadrant crossings, with such crossings increasing somewhat across the days of testing on this phase of the experiment.

Spatial learning set acquisition testing

In this phase, where the goal position is novel on trial one, the correct behavioral response on trial two depends on retaining this information in order to efficiently find the new location of the escape platform. Therefore, the task can be considered sensitive to the behavioral flexibility of the animal [40] and an inability to alter behavior as spatial location escape platform changes, should be reflected in perseverative behavior [41].

In order to facilitate the interpretation of the data, trial one versus two performances on each day was examined across the five-day test period. Following data screening and the choice of the square root transformation, the resulting data using a 1-between (drugs), 2-within (days, trials) mixed ANOVA uncovered the following effects. Main effects of drug groups, $F(1,14)=4.60$, $p<0.05$, $\eta p2=0.247$, days of testing, $F(4,56)=4.05$, $p<0.01$, $\eta p2=0.224$, and trials, $F(4,56)=19.59$, $p<0.01$, $\eta p2=0.583$, were detected. Here, escape latencies were higher among the 5-IAI rats (M=3.90, SD=0.68) than control animals (M=3.11, SD=0.41), escape latencies declined across test days, and escape latencies on trial two were lower than on trial one. However, these results must be considered in light of a significant group X trials interaction, $F(1,14)=6.17$,$p<0.05$, $\eta p2=0.306$. The resulting data are presented in Figure 2, Panel 1. Decomposition of the interaction by comparing the trial one versus two escape latencies for each drug group revealed that in the saline control group, trial two escape latencies were significantly lower than on trial one. On the other hand, the difference between trial one and trial two escape performance among the 5-IAI rats was non-significant. This finding is bolstered by a between-groups comparison within each trial. Here, while escape latencies were comparable on trial one, control rat escape latencies were significantly lower on trial two than that of the 5-IAI rats.

With some notable differences, a similar pattern to the escape latency data was observed when the quadrant errors data was examined (see Figure 2, Panel 2). Here, the main effects of drug group and days were non-significant, while the main effect of trials, $F(1,14)=46.05$, $p<0.001$, $\eta p2=0.767$, and more important, the groups X trials interaction, $F(1,14)=10.97$, $p<0.01$, $\eta p2=0.439$, was significant. While the number of quadrant errors among the 5-IAI rats did not differ between trials one and two, control rats crossed significantly fewer quadrants on trial two than on trial one. However, a between group comparison of the trial two data failed to reach significance ($p=0.053$).

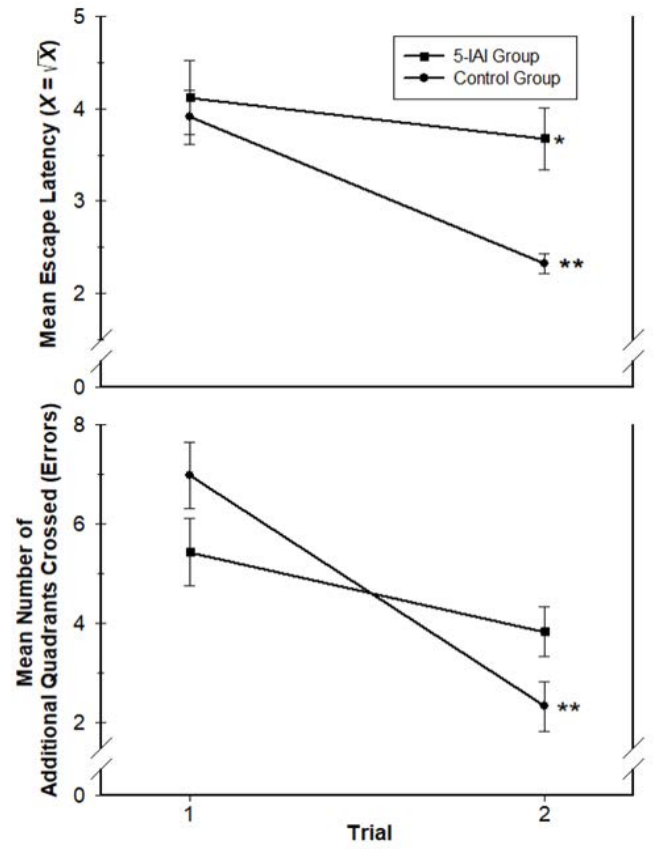

Figure 2) *Graphical representation of the mean and the standard error of the mean (SEM; vertical bars) for trial one versus trial two performances on the spatial learning set task collapsed across the 5-day test period. Vertical lines represent the standard error of the mean (SEM).*

***Significant difference between trial one and trial two escape latencies or number quadrant errors ($p<0.05$). *Significant difference saline and 5-IAI-treated rats ($p<0.05$).*

Neurochemical analysis of brain 5-ht and da levels

Examination of cortical or subcortical 5-HT and DA levels revealed no significant differences between the saline and 5-IAI-treated animals (all $ps>0.05$). Likewise, all measures of 5-HIAA and DOPAC levels were comparable across groups.

DISCUSSION

In the present experiment, the performance of 5-IAI-treated rats was inferior to that of the saline control animals. However, such performance deficits were confined to two presumably more difficult phases of the experiment–the difficult place learning and learning set tasks–with no group differences found in phases with a visible platform or a number of allocentric cues to facilitate navigation. Last, both groups demonstrated comparable passive avoidance learning.

As reported earlier, the 5-IAI-treated rats reared on their hind limbs more often than the control animals. When rats are placed in a novel environment, rearing tends to increase, typically followed by a decline due to environmental habituation [42]. Compounds with stimulant properties such as amphetamine and cocaine can induce increases in rearing behavior [42], and MDMA increases rearing behaviors following abstinence [43]. Often this behavior has been ascribed to aminergic stereotypies [42]. Nonetheless, the rearing behavior is oriented in a manner consistent with incoming airstreams [44], relevant in a species that places a considerable reliance on olfaction.

Like other developmental periods, the time-frame comprising adolescence is one with a striking number of neural changes [17]. Among these are the maturational changes to 5-HT neural systems [18,19], including differential expression of 5-HT receptors with age [20]. Further, considerable evidence of the role of cortical noradrenergic systems studies in executive function have been reported [45-48]. Norepinephrine transporter (NET) changes are found between adolescence and young adulthood in rats (PND 40-60), but such changes are regionally selective [49,50]. Owing to the anatomical and functional transformations associated with adolescence, exposure to drugs during this period can be acutely problematic [21]. An instructive example can be found in the literature on the effects of adolescent exposure to MDMA. Here, using indices as direct neurotransmitter levels, metabolites, and binding sites suggest that MDMA exposure may lead to a long-term decrease in 5-HT levels [22-26].

As noted earlier, MDMA potency is double that observed in 5-IAI [12,13], which may offer a partial explanation for the results reported here including the absence of any reduction in measured monoamine levels. Noradrenergic and serotoninergic transporters are more strongly inhibited among aminoindanes such as 5-IAI and 5,6-methylenedioxy-2-aminoindane (MDAI) [11] but, like MDMA, preferentially promotes 5-HT release through SERT inhibition [12].

While speculative, work with MDMA may suggest another possibility. Using a protocol similar to the one we used in the present study, learning and memory deficits in rats exposed to MDMA during adolescence were found across much of the lifespan even in the absence of differences in 5-HT or DA levels [34]. In another investigation, the effects of MDMA in adolescent rats were explored through multiple subcutaneous dose exposures spaced at five-day intervals from PND 35 to 60 [26]. The results of Piper and Meyer revealed that though the damage to serotonergic systems was largely absent, the prior MDMA exposure compromised cognition. In a follow up study, prior MDMA exposure led to a predicted attenuation of the neurotoxic changes that would otherwise have been expected [51].

Further, Coppola and Mondola [9] offer another possibility worth exploring. As they noted, methamphetamine derivatives are associated with neurotoxicity in part driven by altered activity in glutamatergic systems and neuro-inflammation (e.g., [52-55]). However, no research has been reported that considered the impact of 5-IAI on glutamatergic systems, neuro-inflammation, or oxidative stress [9].

While the results are suggestive that 5-IAI consumption is not without

risk to cognition, the present results must be considered in light of the limitations of the present study as well as any direct relevance to the effects of 5-IAI in humans. Here, given the pilot nature of our investigation, we only considered a single dose. In small animals, drug elimination rates tend to be higher than in larger animals such as humans [53]. Naturally, it is desirable to perform direct mg/kg comparisons. However, it doing so there is a risk leading to underestimating the bioavailability of a given drug [56]. Fortunately, the use of allometric scaling formulas permits interspecies comparisons [57] and interspecies inferences concerned with drugs of abuse-Dosehuman=Doseanimal (Weighthuman/Weightanimal) 0.25, with dose and weight expressed in mg/kg and kg respectively. In addition, an adjustment to the exponent to 2/3 has been suggested [58]. Thus, calculated for a single dose of 5-IAI at 20 mg/kg for a 0.200 kg adolescent rat would be considered equivalent to a dose in a 50 kg adolescent human of approximately 3.234 mg/kg or about 162 mg of drug. This dose is within the range (100 mg–1 gram) reported by recreational users (3-6). When compared to MDMA, considerably much less information concerning the pharmacodynamics of 5-IAI is available but anecdotes by recreational users as well as the work of Nichols and colleagues (2) suggest that our research with the dose reported here has value. Last, it would be worthwhile to consider adolescent exposure of 5-IAI to measures at multiple time points across the rodent lifespan.

In any discussion of developmental exposure, both the timing of and length of exposure are important considerations., For example, in the rodent nucleus accumbens-part of the reinforcement system of the brain [59]–5-HT levels are up to four times lower in rats during PNDs 30 to 40 when compared with prepubescent rats or older (PND 60 to 80) rats [60]. In addition, just before the onset of adolescence, 5-HT2A receptors are at the highest level of expression in the cortex, followed by a decline to that observed at adult levels [61]. As such, framing adolescent exposure of 5-IAI as particularly disconcerting given the possible long-term consequences is worthy of additional examination.

In adult animals, considerable research has gone into elucidating the effects of psycho-stimulant exposure in adult animals. Unfortunately, the examination of such effects using adolescent animals remains incomplete [62]. Bio (behavioral) research on the effects of 5-IAI is largely absent, even though concerns about the abuse of this drug have been reported [10]. When the effects on humans are considered, the number of regulatory and ethical considerations reduces the opportunity for well-designed investigations involving human subjects, including and especially using adolescent subjects. Where found in the literature, they are often compromised by a number of sampling issues as well as confounding variables including polydrug use [62] and the purity of the drugs used. To reiterate, beyond anecdotes, such investigations are completely absent when 5-IAI is considered. Therefore, it is judicious for researchers to continue to examine such emerging drugs of abuse, examining such variables as frequency of exposure, dose, and exposure at various timepoints in the lifespan.

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