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Effects of Designer Drugs on the Chicken Embryo and 1-Day-Old Chicken

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BRONSON, M. E., W. JIANG, C. R. CLARK AND J. DeRUITER. *Effects of designer drugs on the chicken embryo and 1-day-old chicken.* BRAIN RES BULL **34**(2) 143–150, 1994.—The present study was conducted to examine the effects of *d*-amphetamine and the designer drugs 3,4-methylenedioxymethamphetamine (MDMA), *N*-methyl-3,4-methylenedioxyphenyl-3-butanamine (HMDMA), 3,4-methylenedioxyphenyl-2-butanamine (BDB), 3,4-methylenedioxyphenyl-1-ethanamine (MDM1EA) in the chick embryo and the young chicken. HMDMA and MDM1EA had no effect on motility on day 14 of embryogenesis, while MDMA, BDB, and *d*-amphetamine decreased embryonic motility at one or more doses. On day 1 posthatch, chickens were challenged with cumulative injections of water or the same drug that they had received in ova. With the exception of MDM1EA, all of the drugs produced effects such as distress vocalization, wing extension, tremor, flat body posture, bursting forward movements, loss of righting reflex, and convulsant-like kicking. Pretreatment with drug in ova resulted in tolerance to certain drug effects and supersensitivity to other drug effects. Furthermore, BDB significantly decreased hatchability, MDM1EA decreased body weight, and HMDMA decreased liver weight. Further studies are neeeded to determine the mechanism(s) of toxicity in this species.

Designer drugs MDMA ("Ecstasy") MDMA analogues and homologues *d*-Amphetamine Chicken embryo Hepatotoxicity

DRUG abuse during pregnancy has been recognized as a problem since the mid-1960s (18). Today, almost 30 years later, pregnant women are still abusing drugs, as evidenced by an increasing literature on fetal alcohol syndrome, opioid dependence in the neonate, and the effects of maternal cocaine use on the fetus and neonate. Although much is known about the effects of these abused drugs on both the adult and developing organism, less is known about the effects of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") and MDMA analogs and homologs that have turned up in forensic laboratories in the United States and Canada (25). MDMA was originally synthesized in clandestine laboratories by a reductive amination procedure in which commercially available 1-(3,4-methylenedioxyphenyl)-2-propanone (MDP2P) was treated with methylamine in the presence of a reducing agent such as sodium cyanoborohydride. In 1989, however, the Drug Enforcement Administration passed the Chemical Diversion and Trafficking Act that controlled the availability of MDP2P. The restricted availability of MDP2P has prompted clandestine chemists to explore alternative synthetic methods for the production of MDMA. The resulting products include *N*-methyl-3,4-methylenedioxyphenyl-3-butanamine (HMDMA) (23); 3,4-methylenedioxyphenyl-1-ethanamine (MDM1EA); *N*-methyl-3,4-methylenedioxyphenyl-2-butanamine, also known as *N*-methyl-1-(1,3-benzodioxol-5yl)-2-butanamine or MBDB, and 3,4-methylenedioxyphenyl-2-butanamine, also known as 1-(1,3-benzodioxol-5yl)-2-butanamine) or BDB (22). HMDMA has been reported to be weakly spasmolytic (17), and is approximately twice as toxic as MDMA in mice (8). Although some information is available on the effects of MBDB (22), to date, virtually nothing is known about the pharmacological, toxicological, and behavioral effects of BDB. Furthermore, these compounds may be distributed on the streets as MDMA. Recently, in the United Kingdom, several deaths and seven cases of hepatotoxicity in otherwise apparently healthy MDMA users have occurred following recreational use of small amounts of MDMA (13). Reports of MDMA toxicity of this kind are rare and fairly recent, suggesting that the toxicity may not be due to MDMA but rather to some synthetic intermediate or byproduct. There is certainly a precedent for toxicity due to administration of clandestinely manufactured drugs, as evidenced by the appearance of Parkinson's Disease symptoms in persons who had injected a meperidine analogue, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (9).

The purpose of the current study was to examine the effects of MDMA and selected homologs and analogs in the chicken embryo and newly hatched chicken and to compare the effects to those of *d*-amphetamine and water. The chick embryo provides an excellent model for studying direct drug effects on the fetus without potentially complicating maternal factors such as polydrug use, malnutrition, etc. The chick embryo has been used extensively in biochemical, pharmacological, electrophysiological, neuroanatomical and behavioral studies, providing a

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large literature base for comparison. In addition, it is a less expensive preparation for studying direct drug effects on the fetus than the current existing mammalian model, the pregnant ewe (6,36).

MDMA, HMDMA, MDM1EA, BDB, and *d*-amphetamine were tested for effects on spontaneous embryonic motility, which is neurogenic in origin (1,26,27,31) and is a convenient and rather accurate index of CNS motor development (30). Perturbations to the embryonic system, such as injection of a drug, have been shown to alter avian embryonic motility. For example, spontaneous motility is first present on day 4.5 of embryogenesis (1), and injection of morphine on day 5 dose dependently decreased this motility (21). Furthermore, tolerance develops to morphine-induced decreases in embryonic motility (32). Embryonic motility has also been used as a model for acute opioid dependence (3,19). In acute dependence studies, opioids typically decrease motility, whereas subsequent injection of an opioid antagonist significantly increases motility above preopioid baseline, an effect that has been interpreted as precipitated withdrawal. Other drugs that affect motility in the chick embryo are γ-amino-butyrate (GABA) and a $GABA_B$ receptor agonist, baclofen, both of which decreased activity (20), while GABA antagonists such as bicuculline and picrotoxin elicited increases in motility (31). Interestingly, L-DOPA and *d*-amphetamine also decreased motility in the chick embryo (29); this paradoxical effect may be explained by the fact that dopamine appears to be a CNS depressant in birds (12).

The chick embryo has also been used as a model for drug effects on fetal viability, gestation length, and congenital abnormalities. To our knowledge, no studies have been carried out on embryonic exposure to MDMA and related compounds. A literature exists, however, on embryonic exposure to other drugs of abuse. In humans, for example, cocaine use during pregnancy results in higher rates of spontaneous abortion than are seen in nonsubstance abusing patients (5). More recently, it has been reported that there was a significant decrease in birth weight, head circumference, length, and gestational age for offspring of women that tested positive for cocaine, amphetamine, or opioids when compared to offspring of women who had negative drug screens (11). Similar effects can be seen in the chick embryo after administration of drugs. Exposure to methadone and morphine, for example, resulted in decreased liver weight and brain protein, while high doses of methadone resulted in a dose-dependent failure to develop (16). In contrast, lower doses of another opioid, *N*-desmethyl-l-α-acetylmethadol (NLAAM) had no effect on viability, but subsequent exposure to naloxone (i.e., precipitated withdrawal) significantly decreased hatchability (19). Reserpine (33) and methylmercury (14) also resulted in decreased hatchability, as did the solvents acetone, ethylene glycol, and ethanol (2). As early as 1914, it was reported that exposure of the chick embryo to ethanol vapor resulted in developmental retardation and vascular, CNS, and eye defects (35). One purpose of this study was, therefore, to determine whether similar effects would be seen after embryonic exposure to *d*-amphetamine and MDMA and selected homologs and analogs.

The current study also examined the effects of MDMA and its homologs and analogs in the newly hatched chicken because, like the human neonate, the newly hatched chicken is very susceptible to the effects of drugs. For example, sedative compounds such as barbiturates, chlorpromazine, and morphine produced behavioral depression and associated slow wave EEG activity in 5–14-day-old chickens, whereas hallucinogenic compounds such as lysergic acid diethylamide and mescaline caused behavioral excitation, EEG arousal, and abnormal body postures (34). It has also been demonstrated that a single dose of methadone resulted in acute dependence in the 1–3-day-old chicken, as evidenced by signs of withdrawal when naloxone was administered 1–4 h after the initial dose of methadone (4), and the withdrawal signs were similar to those seen in human neonates undergoing opioid withdrawal (10,28). Thus, the young chicken may provide a good model for studying perinatal effects of MDMA and related compounds. Furthermore, the possibility exists that exposure to drug during embryogenesis may lead to tolerance and/or sensitivity to the same drug in the neonate. To test this theory, young chickens were challenged with MDMA and its analogs and homologs after being treated once during embryogenesis with either the same compound or vehicle.

METHOD

Chick Embryo Studies

Animals. Fertile chicken eggs were obtained from a local hatchery. The eggs were kept in a forced air incubator maintained at 37°C and 58% relative humidity. On day 14, eggs were removed from the incubator and candled to determine viability. On the 19th day of incubation eggs were transferred to a forced air hatcher (maintained at 38°C and 58–60% relative humidity).

Drug synthesis. MDMA and homologs and analogs that may appear in street samples sold as MDMA were synthesized as we have reported earlier (24,25), using the methods commonly employed by clandestine chemists. These methods include either reductive amination of commerically available 1-(3,4-methylenedioxyphenyl)ketones or synthesis and reduction of a methylenedioxyphenylnitrostyrene. Thus, treatment of the commercially available ketones 1-(3,4-methylenedioxyphenyl)acetophenone, 2-propanone or 3-butanone with methylamine in the presence of sodium cyanoborohydride yields MDM1EA, MDMA, and HMDMA, respectively. BDB was prepared by reaction of a piperonal imine with nitropropane followed by catalytic reduction of the resultant intermediate methylenedioxynitrostyrene, as we have reported earlier (23). All of the amine products were converted to the corresponding hydrochloride salts by treatment with ethereal HCl. Prior to behavioral and pharmacological testing, the structures of all products were established by standard spectroscopic techniques (IR, NMR, and MS) and purity was confirmed by chromatographic methods and elemental analysis. *d*-Amphetamine was obtained from Sigma Chemical Co., St. Louis, MO.

Drugs and routes of administration. For the chick embryo studies, all compounds were injected directly into the chorioallantois of the egg close to the vitellene vessels, via a small hole drilled in the shell. Water and all drugs were injected on day 14 as this time point of embryogenesis allows for maximal drug effects on motility. This time point approximates the beginning of the third trimester in humans. The drug compounds tested were MDMA, BDB, HMDMA, MDM1EA and *d*-amphetamine. All compounds were dissolved in distilled water and injection volume was 0.05 ml/egg. Doses, expressed as the salt, were 8, 16 and 32 mg/kg egg. These doses were chosen because they are similar to the doses of *d*-amphetamine that produced decreases in motility in another study (29), thus allowing for comparison. The doses of MDMA and related compounds were selected so that a direct comparison could be made with *d*-amphetamine and also because the dose range encompasses the doses of MDMA (10–20 mg/kg) that have been used to produce neurotoxicity in rats. HMDMA 4 mg/kg egg was also tested. To provide contemporaneous controls, there was a water group for each drug group.

Motility recording. Platinum subdermal electrodes (type E2, Grass Instruments Co., Quincy, MA) connected to a Physiograph Four-B recorder (Narco Biosystems, Inc., Houston, TX) were used to conduct the electrical potential produced by movements of the fetus (15) before and after delivery of vehicle or drug. The egg was placed on a foam rubber cup and electrodes were inserted approximately 2 mm into each of two holes drilled approximately 180° apart midway along the longitudinal axis of the egg. The signal was amplified by a Channel Amplifier 7000, which was calibrated daily such that a 1 mV calibration signal produced a 4.0 cm recorder pen deflection above the baseline recording from a nonviable egg. The physiograph paper advanced at 2.5 mm/s such that motility recordings could be summarized by counting the number of 2 s intervals, aka epochs (equal to one 5 mm division on the physiograph paper) during which the recorder pen was deflected at least 2 cm above baseline. The number of epochs during each testing period was counted and used for statistical analysis. Motility was recorded for 6 min (baseline), after which drug or an equal volume of water was injected directly into the egg. Six minutes later an additional 6 min of motility were recorded (treatment). A pretreatment time of 6 min was chosen because the effects of *d*-amphetamine were most evident at this time point.

Data Analysis

Motility was analyzed by repeated-measures ANOVA followed by post hoc Dunnets' tests with a significance level set at 0.05.

Effects of Drugs on Newly Hatched Chickens

Injection procedure. One-day-old chickens that had been treated in ova with either MDMA, HMDMA, MDM1EA, BDB, *d*-amphetamine, or an equal volume of water were weighed and injected IP with cumulative doses of the same drug that they had received in ova, or with repeated injections of water. Injection volume was 1 ml/kg. Cumulative doses of drugs were 4, 8, 16, and 24 mg/kg. There were four groups of chicks: 1) those that received drug in ova, challenged with drug after hatch, 2) those that received drug in ova, challenged with vehicle after hatch, 3) those that received vehicle in ova, challenged with vehicle after hatch, and 4) those that received vehicle in ova, challenged with drug after hatch. The injection regimen was as follows: observe chickens for 5 min and test for loss of righting reflex; inject first dose, then observe for 5 min; inject second dose and observe for an additional 5 min, etc. Groups of four chicks at a time were observed following each injection, by an investigator who was blind to treatment regimen. Chickens were observed immediately after injection because, in contrast to drug effects on motility, which were seen only after several minutes, effects on the newly hatched chicken appear within 1 min, thus leaving an additional 4 min for observation. Signs such as distress vocalization, outstretched wings, flat body posture, bursting forward movements, loss of righting reflex, and convulsant-like kicking were scored as either present or absent. Chickens were judged to have lost their righting reflex if they could not right themselves within a 30 s period after being placed on their backs. After observing the effects of drug or vehicle injection, chickens were euthanized with CO_2 and their livers and brains were dissected out and weighed.

Data Analysis

Body, brain, and liver weight were analyzed by two-factor ANOVA and Fishers LSD post hoc tests with a significance level set at 0.05. Hatching data and scoring of drug symptoms were analyzed by chi square, after which confidence intervals for binomial probability were determined.

RESULTS

Motility

The effects of MDMA, HMDMA, MDM1EA, BDB, and *d*-amphetamine on motility in the 14-day-old embryo are summarized in Table 1. Water had no effect on motility when compared to preinjection baseline. Similarly, MDM1EA and HMDMA had no effect on motility at doses ranging from 8 to 32 mg/kg egg. Repeated-measures ANOVA revealed a repeated-measures effect in the *d*-amphetamine group $F(1, 3) = 5.223$, $p = 0.0241$. Post hoc analysis by Dunnett's test revealed that the effect was due to a decrease in motility following the 8 and 16 mg/kg doses of *d*-amphetamine, $p < 0.05$. There was also a repeated-measures effect in the MDMA group $F(1, 3) = 5.135$, $p = 0.0259$, and this effect was due to a decrease in motility following the highest dose of MDMA, 32 mg/kg, $p < 0.005$. BDB also showed a repeated-measures effect $F(1, 3) = 4.148$, $p = 0.045$, but this effect occurred at the intermediate, 16 mg/kg, dose of BDB and was also manifest as a decrease in motility, $p < 0.05$.

Drug Effects on Behavior 1 Day Posthatch

Tables 2–5 show the effects of MDMA, HMDMA, BDB, and *d*-amphetamine, respectively in 1-day-old chickens that were

TABLE 1

EFFECTS OF WATER, MDMA, HMDMA, MDM1EA, BDB, AND *d*-AMPHETAMINE ON MOTILITY IN THE 14-DAY-OLD CHICK EMBRYO

		Epochs per 2 Min (Mean ± SE)	
Treatment (mg/kg)	(*n*)	Baseline	Treatment
Water	20	23.4 (2.17)	22.9 (1.87)
MDMA (8)	30	19.6 (2.00)	17.6 (1.71)
MDMA (16)	22	15.8 (2.04)	15.5 (2.48)
MDMA (32)	19	17.1 (3.17)	11.0 (2.39)*
Water	32	26.6 (3.04)	22.2 (2.79)
HMDMA (4)	18	25.6 (4.06)	24.2 (3.80)
HMDMA (8)	24	22.7 (3.16)	23.6 (3.00)
HMDMA (16)	38	21.1 (2.65)	21.6 (2.46)
HMDMA (32)	32	23.2 (2.63)	24.4 (2.89)
Water	28	21.5 (2.37)	16.2 (2.41)
MDM1EA (8)	37	23.6 (2.43)	20.1 (2.52)
MDM1EA (16)	43	23.6 (2.39)	24.1 (2.62)
MDM1EA (32)	34	22.1 (2.5)	23.8 (2.68)
Water	18	22.3 (3.61)	22.3 (2.75)
BDB (8)	22	21.6 (3.22)	18.9 (3.50)
BDB (16)	21	23.0 (3.16)	17.3 (2.65)*
BDB (32)	22	20.9 (3.21)	20.3 (2.56)
Water	30	22.6 (2.07)	24.4 (2.47)
d-Amphetamine (8)	33	20.4 (3.16)	14.9 (2.75)*
d-Amphetamine (16)	30	20.6 (2.47)	14.6 (2.58)*
d-Amphetamine (32)	26	25.9 (3.17)	24.5 (3.40)

Baseline motility was recorded for 6 min, after which drug was injected. Six minutes after injection, an additional 6 min of motility were recorded. Data are expressed as mean (±SE) epochs per 2 min period.

* Significantly different from baseline, $p < 0.05$.

TABLE 2

EFFECTS OF WATER OR A CUMULATIVE DOSE OF 24 mg/kg MDMA, ON 1-DAY-OLD CHICKENS PRETREATED ON DAY 14 IN OVA WITH EITHER WATER OR MDMA 8, 16, OR 32 mg/kg EGG

Treatment In Ova	Posthatch Treatment	Number of Chickens Showing Sign/Total Number of Chickens						
		DV	WE	T	FBP	B	LRR	K
Water	MDMA-24 mg/kg	10/10*	10/10*	0/10	6/10*	4/10*	1/10	0/10
MDMA 8 mg/kg	MDMA-24 mg/kg	9/10*	10/10*	0/10	10/10*†	4/10*	2/10	0/10
MDMA 16 mg/kg	MDMA-24 mg/kg	10/10*	10/10*	0/10	10/10*†	5/10*	7/10*†‡	0/10
MDMA 32 mg/kg	MDMA-24 mg/kg	10/10*	10/10*	0/10	0/10†	3/10	0/10	0/10
Water	Water	2/13	0/13	0/13	0/13	0/13	0/13	0/13
MDMA 8 mg/kg	Water	0/9	3/9	0/9	0/9	0/9	0/9	0/8
MDMA 16 mg/kg	Water	0/10	3/10†	0/10	0/10	0/10	0/10	0/10
MDMA 32 mg/kg	Water	0/10	2/10	2/10	0/10	0/10	0/10	0/

DV = distress vocalization, WE = wing extension, T = tremor, FBP = flat body posture, B = bursting forward movements, LRR = loss of righting reflex, K = convulsant-like kicking. Data are presented as a ratio of the number of chickens expressing a particular sign to the total number of chickens in each treatment group.

* Different from water pretreatment in ova after same posthatch injection, $p < 0.05$.

† Different from posthatch water injection after same pretreatment in ova, $p < 0.05$.

‡ Different from pretreatment with MDMA 8 and 32 mg/kg, $p < 0.05$.

treated on day 14 in ova with either water or the same drug injected posthatch. For clarity, because there are at least eight treatment groups per drug and each chicken received three to four injections posthatch, only the effects of the highest cumulative dose are shown. Data for MDM1EA are not presented in a table because of general lack of effect with this particular drug.

MDMA. Table 2 shows the effects of water and 24 mg/kg MDMA on chickens treated on day 14 in ova with water or 8, 16, or 32 mg/kg egg MDMA. When compared to chickens injected with water posthatch, MDMA produced significantly more distress vocalization and wing extension in all pretreatment groups. MDMA also produced flat body posture and bursting forward movements in chickens pretreated in ova with water or 8 or 16 mg/kg egg MDMA, and these effects were significantly greater in the MDMA pretreated animals as compared to water-pretreated animals. In contrast, these effects were not reliably seen in chickens pretreated with 32 mg/kg egg MDMA. MDMA also produced loss of righting reflex in chickens pretreated with 16 mg/kg egg MDMA, an effect rarely seen after water-pretreatment or pretreatment with 8 or 32 mg/kg egg MDMA. Interestingly, water injections in chickens pretreated with 16 mg/kg egg MDMA also produced wing extension, but this effect was significantly less than that seen when MDMA was administered to chickens that had received the same dose of MDMA in ova.

HMDMA. As seen in Table 3, chickens treated in ova with 4, 8, or 32 mg/kg HMDMA were more susceptible to HMDMA-induced distress vocalization and wing extension than chickens exposed to water or HMDMA 16 mg/kg in ova. In the case of animals pretreated with HMDMA 8 mg/kg, however, water injections posthatch also resulted in wing extension that was not different from wing extension after injection of 24 mg/kg HMDMA. Both water and HMDMA injections posthatch resulted in tremors in animal pretreated with 8, 16, or 32 mg/kg egg HMDMA. In contrast, HMDMA, but not water, produced tremors in water-pretreated controls. HMDMA also resulted in loss of righting reflex in water-pretreated animals and animals

TABLE 3

EFFECTS OF WATER OR A CUMULATIVE DOSE OF 24 mg/kg HMDMA, ON 1-DAY-OLD CHICKENS PRETREATED ON DAY 14 IN OVA WITH EITHER WATER OR HMDMA 4, 8, 16, OR 32 mg/kg EGG

Treatment In Ova	Posthatch Treatment	Number of Chickens Showing Sign/Total Number of Chickens						
		DV	WE	T	FBP	B	LRR	K
Water	HMDMA-24 mg/kg	1/18	3/18	11/18*	0/18	0/18	5/18*	0/18
HMDMA 4 mg/kg	HMDMA-24 mg/kg	5/10*†	7/10*†	0/10†	0/10	0/10	0/10†	0/10
HMDMA 8 mg/kg	HMDMA-24 mg/kg	7/12†	8/12†	8/12	0/12	0/12	5/12	0/12
HMDMA 16 mg/kg	HMDMA-24 mg/kg	3/10	3/10	4/10	0/10	0/10	1/10	0/10
HMDMA 32 mg/kg	HMDMA-24 mg/kg	5/11*†	6/11*†	5/11	0/11	0/11	2/11	0/11
Water	Water	2/13	0/13	0/13	0/13	0/13	0/13	0/13
HMDMA 4 mg/kg	Water	1/10	3/10	1/10	0/10	0/10	0/10	0/6
HMDMA 8 mg/kg	Water	4/18	6/18†	7/18†	0/18	0/18	4/18†	0/18
HMDMA 16 mg/kg	Water	0/10	2/10	3/10†	0/10	0/10	1/10	0/10
HMDMA 32 mg/kg	Water	0/10	0/10	3/10†	0/10	0/10	0/10	0/10

Other details are the same as in Table 2.

* Different from posthatch water injection after same pretreatment in ova, $p < 0.05$.

† Different from water pretreatment in ova after same posthatch injection, $p < 0.05$.

TABLE 4

EFFECTS OF WATER OR A CUMULATIVE DOSE OF 16 mg/kg BDB, ON 1-DAY-OLD CHICKENS PRETREATED ON DAY 14 IN OVA WITH EITHER WATER OR BDB 8, 16, OR 32 mg/kg EGG

		Number of Chickens Showing Sign/Total Number of Chickens						
Treatment In Ova	Posthatch Treatment	DV	WE	T	FBP	B	LRR	K
Water	BDB 16 mg/kg	12/12*	12/12*	6/12*	12/12*	12/12*	12/12*	0/12
BDB 8 mg/kg	BDB 16 mg/kg	10/10*	10/10*	2/10†	7/10*	5/10*	6/10*	0/10
BDB 16 mg/kg	BDB 16 mg/kg	10/10*	10/10*	0/10†	8/10*	7/10*	6/10*	0/10
BDB 32 mg/kg	BDB 16 mg/kg	10/10*	10/10*	0/10†	5/10*	4/10*	3/10*	0/10
Water	Water	1/13	2/13	0/13	0/13	0/13	0/13	0/13
BDB 8 mg/kg	Water	4/12	2/12	0/12	0/12	0/12	0/12	0/12
BDB 16 mg/kg	Water	1/15	3/15	1/15	0/15	0/15	0/15	0/9
BDB 32 mg/kg	Water	2/10	3/10	0/10	0/10	0/10	0/10	0/10

Other details are the same as in Table 2.
* Different from posthatch water injection after same pretreatment in ova, $p < 0.05$.
† Different from water pretreatment in ova after same posthatch injection, $p < 0.05$.

pretreated with 8 mg/kg egg HMDMA. In the case of chickens pretreated with 8 mg/kg egg HMDMA, however, water injections also produced loss of righting reflex; thus, this treatment group appeared to be more sensitive overall to the injection procedure.

MDM1EA. In general, MDM1EA had no effect on behavior, regardless of pretreatment in ova. The only exception was that animals treated with 32 mg/kg MDM1EA in ova were more susceptible to tremors after injection of water posthatch.

BDB. Table 4 shows that when compared to water injections, 16 mg/kg BDB produced significantly more distress vocalization, wing extension, flat body posture, bursting movements, and loss of righting reflex, regardless of pretreatment in ova. BDB also produced significantly more tremors in water-pretreated chickens than in BDB pretreated chickens.

d-*Amphetamine.* The effects of *d*-amphetamine are summarized in Table 5. Like BDB, *d*-amphetamine produced significantly greater distress vocalization, wing extension, tremor, flat body posture, and loss of righting reflex when compared to water injections. In contrast to BDB, *d*-amphetamine did not produce bursting movements but, instead, resulted in convulsant-like kicking. Furthermore, chickens pretreated with 8 or 16 mg/kg egg *d*-amphetamine in ova were more sensitive to *d*-amphetamine-induced kicking when compared to water-pretreated chickens, and chickens pretreated with 8 mg/kg egg *d*-amphetamine also showed increased tremor when compared to water-pretreated chickens.

Hatchability

Hatchability was approximately 90% in all groups except the 32 mg/kg egg BDB group. In this latter group, hatchability was less than 40%. The decrease in hatchability was due to the fact that the embryos died within a few days following this high dose of BDB, not to the fact that they were too weak to hatch.

Weights at Hatch

The effects of the various drugs on body, brain, and liver weight are summarized in Table 6.

Body weight. Two-factor ANOVA revealed a significant treatment effect on body weight at hatch in the HMDMA and MDM1EA groups, $F(4, 112) = 5.161$, $p = 0.0007$ and $F(3, 76) = 3.082$, $p = 0.0323$, respectively. Fishers PLSD revealed that the difference was due to significantly increased body weights in chickens that had received HMDMA 4 and 8 mg/kg egg and decreased body weights in the MDM1EA 32 mg/kg egg pretreat-

TABLE 5

EFFECTS OF WATER OR A CUMULATIVE DOSE OF 16 mg/kg *d*-AMPHETAMINE, ON 1-DAY-OLD CHICKENS PRETREATED ON DAY 14 IN OVA WITH EITHER WATER OR *d*-AMPHETAMINE 8, 16, OR 32 mg/kg EGG

		Number of Chickens Showing Sign/Total Number of Chickens						
Treatment In Ova	Posthatch Treatment	DV	WE	T	FBP	B	LRR	K
Water	*d*-Amphetamine 16 mg/kg	9/10*	9/10*	6/10*	9/10*	0/10	10/10*	4/10*
d-Amp 8 mg/kg	*d*-Amphetamine 16 mg/kg	10/10*	10/10*	10/10*†	10/10*	0/10	10/10*	10/10*†
d-Amp 16 mg/kg	*d*-Amphetamine 16 mg/kg	9/10*	9/10*	7/10*	9/10*	0/10	8/10*	9/10*†
d-Amp 32 mg/kg	*d*-Amphetamine 16 mg/kg	7/9*	8/9*	6/9*	6/9*	0/9	6/9*	6/9*
Water	Water	2/9	0/9	0/9	0/9	0/9	0/9	0/9
d-Amp 8 mg/kg	Water	0/10	3/10	0/10	0/10	0/10	0/10	0/10
d-Amp 16 mg/kg	Water	0/11	1/11	0/11	0/11	0/11	0/11	0/11
d-Amp 32 mg/kg	Water	0/10	1/10	0/10	0/10	0/10	0/10	0/10

Other details are the same as in Table 2.
* Different from posthatch water injection after same pretreatment in ova, $p < 0.05$.
† Different from water pretreatment in ova after same posthatch injection, $p < 0.05$.

TABLE 6

EFFECTS OF WATER, MDMA, HMDMA, MDM1EA, BDB, AND *d*-AMPHETAMINE (*d*-AMP), ADMINISTERED ON DAY 14 IN OVA, ON BODY WEIGHT AT HATCH, AS WELL AS BRAIN AND LIVER WEIGHTS, EXPRESSED AS PERCENT OF BODY WEIGHT

Treatment in ova (mg/kg)	*n*	Body Weight (g)	Brain (% b. wt.)	Liver (% b. wt.)
Water	23	39.35 (0.682)	2.043 (0.061)	2.630 (0.102)
MDMA (8)	20	40.20 (0.613)	1.998 (0.037)	2.531 (0.103)
MDMA (16)	24	38.42 (0.874)	2.042 (0.052)	2.719 (0.136)
MDMA (32)	22	39.41 (0.538)	2.014 (0.038)	2.704 (0.094)
Water	21	36.45 (0.579)	2.179 (0.046)	2.905 (0.063)
HMDMA (4)	19	41.08 (0.734)*	2.038 (0.037)	2.395 (0.067)*
HMDMA (8)	32	38.41 (0.548)*	2.018 (0.040)	2.458 (0.058)*
HMDMA (16)	21	37.31 (0.933)	2.107 (0.059)	2.597 (0.078)*
HMDMA (32)	24	38.25 (0.749)	2.131 (0.057)	2.633 (0.085)*
Water	20	40.18 (0.929)	1.976 (0.070)	2.515 (0.134)
MDM1EA (8)	24	38.63 (0.461)	2.046 (0.030)	2.569 (0.059)
MDM1EA (16)	19	38.97 (0.960)	1.905 (0.070)	2.477 (0.081)
MDM1EA (32)	17	36.59 (0.924)*	2.077 (0.091)	2.814 (0.127)
Water	20	41.30 (0.852)	1.933 (0.04)	2.418 (0.082)
BDB (8)	21	42.05 (0.738)	1.993 (0.037)	2.499 (0.093)
BDB (16)	25	40.72 (0.593)	1.959 (0.042)	2.478 (0.066)
BDB (32)	20	42.00 (0.652)	2.001 (0.040)	2.580 (0.083)
Water	19	39.18 (0.792)	2.086 (0.069)	2.673 (0.113)
d-amp (8)	22	39.75 (0.610)	2.082 (0.035)	2.493 (0.067)
d-amp (16)	21	39.52 (0.867)	1.978 (0.058)	2.439 (0.077)
d-amp (32)	19	39.95 (0.688)	1.987 (0.048)	2.538 (0.064)

* Significantly different from water pretreatment, $p < 0.05$.

ment group when compared to contemporaneous water-injected controls, $p < 0.05$.

Brain weight. There was no difference in brain weights among the various pretreatment groups.

Liver weight. Two-factor ANOVA revealed a significant treatment effect on liver weight in the HMDMA group, $F(4, 112) = 7.3365$, $p = 0.0001$. Fisher's PLSD showed that all doses of HMDMA resulted in decreased liver weights, expressed as percent of body weight, when compared to contemporaneous water-injected controls, $p < 0.05$.

DISCUSSION

In the present study, 8 and 16 mg/kg egg *d*-amphetamine decreased motility in the 14-day-old chicken embryo. These results are similar to those of others (29), who also reported a decrease in motility after administration of *d*-amphetamine. In contrast, the highest dose of *d*-amphetamine had no effect on motility. This finding may reflect a counteractive, nonselective effect of *d*-amphetamine at high doses. MDMA and BDB also decreased motility, with the highest dose of MDMA being equipotent to the lowest dose of *d*-amphetamine, and with BDB being somewhat more potent than MDMA. HMDMA and MDM1EA, in contrast, had no effect on motility at the doses tested. All of these compounds are structurally related; therefore, the current study provides evidence that subtle structural changes can have dramatic effects on the activity of the resulting compound.

With the exception of MDMIEA, all of the compounds tested had visible effects on the 1-day-old chicken. In contrast to the effects of these drugs on motility, administration of the same compounds posthatch resulted first in behavioral excitation, followed by behavioral depression. MDMA typically produced distress vocalization, wing extension, flat body posture, and forward bursting movements, regardless of pretreatment in ova, although chickens pretreated with 8 or 16 mg/kg egg MDMA had a greater incidence of flat body posture than water-pretreated chickens, and chickens pretreated with 32 mg/kg egg MDMA did not display flat body posture at all. Thus, it appears that there was tolerance to this effect in the high dose group and supersensitivity in the two lower dose groups. Chickens pretreated with 16 mg/kg egg MDMA also had significantly increased loss of righting reflex after a posthatch injection of MDMA when compared to all other pretreatment groups. One possible explanation for the decreased effect of MDMA in the high dose group may be that adaptive changes following this dose of MDMA in ova prevented the expression of some of the effects of MDMA posthatch. Alternatively, the apparent supersensitivity seen after pretreatment with 16 mg/kg MDMA and the apparent tolerance seen after pretreatment with 32 mg/kg egg MDMA may be due to residual drug in the yolk which is absorbed by the chicken just prior to hatch. If enough of the 32 mg/kg egg dose was still present in the yolk, then an additional 16 mg/kg MDMA may have resulted in a nonselective effect that prevented the expression of certain signs. This might also explain why the 16 mg/kg egg MDMA group had significantly more loss of righting reflex than the 8 mg/kg egg MDMA and water groups, i.e., when combined with residual MDMA in the yolk, the additional 24 mg/kg posthatch may have been sufficient to produce loss of righting reflex in the group pretreated with 16 mg/kg egg MDMA but not in the group pretreated with the lower, 8 mg/kg egg, dose of MDMA, and, therefore, not in the water-pretreated group either.

In contrast to MDMA, the only effects of HMDMA in water-pretreated animals were tremor and loss of righting reflex. When compared to water-pretreated animals, animals pretreated with HMDMA also had distress vocalization and wing extension after posthatch HMDMA. The group pretreated with HMDMA 8 mg/kg egg was unusual in that they also exhibited distress vocalization, wing extension, tremor, and loss of righting reflex after water injections. This group also experienced more loss of righting reflex after posthatch HMDMA than the other HMDMA-pretreated groups. Because the 8 mg/kg egg HMDMA-pretreatment group was so sensitive to the injection procedure itself, it is impossible to tell whether HMDMA posthatch actually had an effect in this group like it did in the other HMDMA pretreatment groups. Thus, the only conclusion that can be made is that, in general, animals pretreated with HMDMA were more sensitive to HMDMA-induced distress vocalization and wing extension than animals injected with water posthatch.

BDB and *d*-amphetamine were the most potent drugs tested in the 1-day-old chicken, with both drugs producing distress vocalization, wing extension, tremor, flat body posture and loss of righting reflex at a cumulative dose of 16 mg/kg vs. 24 mg/kg of the other drugs. It is noteworthy that exposure to BDB in ova resulted in tolerance to the tremorigenic effects of BDB posthatch. As other effects of BDB occurred in both water and BDB pretreated chickens, it is unlikely that the other effects of BDB prevented the expression of tremor. There were also differences in animals pretreated with 8 and 16 mg/kg egg *d*-amphetamine when compared to water and the 32 mg/kg egg *d*-amphetamine pretreatment group, in that kicking was significantly greater in the former groups. These results are similar to those seen with MDMA, and the explanation for this effect may be the same as that hypothesized for MDMA above. Despite the similar effects seen with BDB and *d*-amphetamine, these drugs differed from each other, in that BDB produced bursting forward movements,

whereas *d*-amphetamine–treated chickens remained in a prone position and exhibited convulsant-like kicking movements. Interestingly, the effects of *d*-amphetamine were identical to those seen in 1-day-old chickens experiencing precipitated opioid withdrawal (4). Experiments are currently under way in this laboratory to determine which neurotransmitters are involved in the effects of *d*-amphetamine and the other compounds tested in the present study.

None of the drugs, at the doses administered in ova, had a significant effect on hatchability when compared to water injections except the highest (32 mg/kg egg) dose of BDB which was lethal in approximately 60% of the embryos. Virtually nothing is known about this compound, but the present results suggest that it may be a very dangerous drug if taken during pregnancy. Furthermore, because increased clandestine synthesis of MDMA derivatives has resulted in compounds such as BDB, it is possible that this drug could appear on the streets as MDMA.

The only compounds that affected hatch weight when compared to water-pretreated controls were MDM1EA (32 mg/kg egg) and the two lowest (4 and 8 mg/kg egg) doses of HMDMA. MDM1EA decreased hatch weight, however, whereas chickens pretreated with HMDMA had increased weights at hatch. Furthermore, HMDMA resulted in decreased liver weight at all doses administered in ova. HMDMA has previously been shown to be more toxic than MDMA (8,17), and the current results support this finding. It is also interesting to note that hepatotoxicity has recently been reported in otherwise healthy MDMA users (13), and that such toxicity has not been reported in the past with MDMA. The authors (13) speculated that the MDMA-related hepatotoxicity may, in fact, have been due to ingestion of a synthetic intermediate or byproduct. In view of the present results with HMDMA, it is possible that what was being sold on the streets as MDMA in the United Kingdom was, in fact, a hepatotoxic derivative of MDMA.

Taken as a whole, the results of the present study indicate that designer drugs such as MDMA and its homologs and analogs may have direct effects on the fetus and neonate, and that certain drugs, such as BDB, may affect embryonic viability. With the increase in clandestine synthesis and use of designer drugs today, there is a need for public awareness of the inherent dangers in these drugs, particularly during pregnancy. Furthermore, studies such as these can be useful in determining potential treatment for neonates exposed to these compounds. To this end, experiments are currently underway in our laboratory to determine what neurotransmitter systems are involved in the effects of these drugs. Preliminary data suggest that both dopamine and serotonin are involved in the posthatch effects of these drugs, as haloperidol, a dopamine antagonist, and ketanserin, a 5-HT_2 antagonist, prevent some of the effects and ketanserin reverses certain effects (unpublished results). Future directions include administration of these antagonists in combination with HMDMA and BDB in ova in an attempt to prevent the hepatotoxicity and lethality, respectively, of these two compounds.

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