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Aggressive Behavior and Jamming Avoidance Response in the Weakly Electric Fish *Gymnotus carapo*: Effects of 3,4-Methylenedioxymethamphetamine (MDMA)

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ABSTRACT. Jamming avoidance response (JAR) and aggressive behavior were evaluated in pairs of weakly electric fish *Gymnotus carapo*. JAR consists of brief frequency increases of the electric organ discharge (EOD) of the faster fish of the pair when a coincidence between the EODs is close to occur. Aggressive behavior is known to be very strong and robust in this species. 3,4-Methylenedioxymethamphetamine (MDMA) treatment significantly decreased the intensity of aggressive behavior while it increases the intensity and probability of occurrence of JAR. After MDMA treatment, the aggressive behavior was replaced by a distinct nonaggressive ("friendly") type of interaction. In addition to JAR, other behaviors, such as novelty response to visual stimuli, were enhanced with MDMA treatment, suggesting that the effects of this drug on aggressive behavior were not due to a general depression of all behavioral displays. Comparative aspects of the described behavioral changes with the effects of MDMA in humans are discussed. COMP BIOCHEM PHYSIOL 118A;3:831–840, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. JAR, aggression, novelty response, MDMA, electric fish, *Gymnotus carapo*, cross correlation, empathy, behavior

INTRODUCTION

The pulse type weakly electric fish *Gymnotus carapo* emits brief electric pulses (4–6 msec) separated by much longer intervals (about 40 msec) of high regularity (0.02 coefficient of variation) (7). The fish can sense transepidermal currents induced both by its own discharge or from external sources, such as the discharge of a cospecific individual, by means of electroreceptors distributed all over its skin (9). The main roles of this electrosensory system are active electrolocation and electrocommunication (3,6,20,21,25,26).

This species exhibits a very intense and robust aggressive behavior that has been described in considerable detail (4,35,36) being probably the most aggressive of all weakly electric fish species. According to Black-Cleworth (4), hierarchical behavior is striking in a two-fish interaction, while territorial behavior becomes more important when more than three individuals cohabit in the same aquarium.

The role of the active electroreception system in the social communication function, including agonistic interactions, is considered to be of great importance. Thus, mean discharge frequencies of individuals are positively correlated with social status in dominance hierarchies (37).

The frequency and rhythm of the electric organ discharge (EOD) sustain several behaviors, such as the novelty response (NR) and jamming avoidance response (JAR). NR consists of a transient frequency increase in response to sensory novelties, which adapts in a few seconds and habituates when the stimulus is repeated. It has been proposed that this behavior bears an exploratory function (5,8,15). JAR consists of modifications in the firing patterns, which serve to minimize detrimental effects on the ability to electrolocate objects due to the interference between the animal's own EOD and those of its neighbors. JARs do not show adaptation or habituation as is the case for NR. The best understood type of JAR behavior is the one shown by the wave type Gymnotiform *Eigenmannia*. In this species, the EOD is a high frequency (mean value 400 Hz) nearly sinusoidal signal. The animal lowers its EOD frequency in response to a sinusoidal signal of higher frequency than its

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Abbreviations–EOD, electric organ discharge; NR, novelty response; JAR, Jamming avoidance response; MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, serotonin.

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own, and raises its EOD rate in response to a signal of lower frequency. This results in an enhancement of the frequency difference (Δf) between the discharges of both fishes, shifting this value to an optimal point where electrolocation performance is not disturbed (15,17,26).

Electrolocation abilities of pulse type Gymnotiforms are vulnerable to a train of foreign pulses that coincides with several successive pulses of their own EODs. Single coincidences are less detrimental (15,16). In these species, a JAR strategy different from that observed in wave type species has been reported (1,2,15,16,18). As the EODs of a pair of fish drift toward a coincidence, the two animals can minimize the number of consecutive pulses coinciding with those of the neighbor in the following way: the faster fish may briefly shorten its EOD interval when a temporal coincidence is close to occur and, simultaneously, the slower fish may briefly stretch its EOD interval. Nevertheless, in some Gymnotiform pulse species decelerations are almost absent. This JAR strategy in pulse species is considered to be an evolutionary predecessor of the JAR in wave species (16).

In a previous study we have demonstrated the possibility of modulating pharmacologically some behavioral responses in this species by different treatments that affect serotonergic neurotransmission (8). The ring-substituted phenylisopropylamine 3,4 Methylenedioxymethamphetamine (MDMA, also known as "Ecstasy") has been reported to have a unique psychoactive profile in humans. MDMA seems to reduce motivation for aggression and enhance empathy (10,14). These characteristics have led to MDMA being considered as the prototypical compound of a new drug class called "entactogens" (27,28).

In the present research we made an attempt to further characterize JAR in *Gymnotus carapo*. Additionally we assessed the effects of MDMA upon JAR, NR and aggressive behavior in this species.

MATERIALS AND METHODS

Animals

Approximately 100 fish weighing 5–50 g were used. Animals were gathered from a lake in Southeastern Uruguay (Laguna del Sauce, Departamento de Maldonado). They were classified as *Gymnotus carapo* both by their external phenotypic characteristics and the head-to-tail multiphasic EOD waveform (34). They were maintained in individual tap water tanks (conductivity: approximately 300 μS) with hiding places composed of plants and a plastic tube; feed consisted of Tubifex worms; water temperature was held at 18°C ± 1°C and lighting was a fluorescent-incandescent lamp commanded by a circadian clock 12L:12D. All experimental procedure were performed during the light phase of the cycle (between 13:30 and 18:00 hr). In every experiment involving pairs of interacting fish, both members of the pairs did not differ more than 5% in weight and length.

Although the sex of the animals was not assessed in this study, almost all fish pairs displayed a clear aggressive behavior under control conditions.

Assessment of Interaction Between Two Fish by EOD Recording

The involvement of the EOD rhythm in social interaction was evaluated by recording the EODs of both animals *isolated* in two different tanks (40 × 30 × 30 cm) followed by the same sequence repeated with the animals *interacting* in the same tank in a lateral antiparallel position with a 2 cm layer of water separating the fish. In these experiments each fish was placed inside a 2.5 cm diameter fenestrated plastic tube adjusted in length to about 1 cm longer than the fish to restraint its movements. The tube had carbon electrodes at both ends to record the EOD.

The EODs of each pair of fish were monitored on an oscilloscope. Each signal was separately filtered with a Schmidt-Trigger circuit with its output (a 5V–0.2 msec pulse) connected to the digital input of a LabMaster 100 DMA card of a PC programmed to sample (at 6 kHz) and calculate point processes of time (PROPUNT 4.2, developed by R. Budelli and R. Saa).

Under control conditions 1–5 min recordings were obtained for each fish pair. Three days later, the same procedure was repeated with the two fishes injected 1 hr before the interaction, with either MDMA (1 mg/kg and 5 mg/kg), or saline. A volume of 5 ml/kg in each case, was injected into the dorsal muscle group.

Additionally, the mean interval of EOD and its standard deviation were assessed individually for each fish immediately before treatment.

Novelty Response

A different sample of animals was assessed for novelty response amplitude (electric, visual, and acoustic sensory modalities) before and 1 hr after MDMA treatment (5 mg/kg). NR amplitude was calculated as the difference (msec) between the resting interval duration (defined as the mean interval of the last 30 sec before the stimulus, with the fish at rest) and the minimal interval reached at the response peak after the stimulus onset. The procedures followed to produce the stimulus of the three sensory modalities mentioned above have been described elsewhere (8).

Behavioral Assays

EVALUATION OF AGGRESSIVE BEHAVIOR. Immediately after the EOD recordings, both on control and after treatment, each fish pair was allowed to interact freely in a glass tank (40 × 30 × 30 cm, with tap water at 18°C ± 1°C). The tank was new for both animals and the interactions began when a separating glass plate was removed. The first

5 min of behavioral display were recorded on a videotape used for further analysis.

Aggression scores were assigned to each interaction by analysis of the tapes. The appearance and intensity of the following aggressive units were evaluated: bites, serpentining, approaches, and jaw locks (4). Each interaction was scored according to an intensity scale (0–3) for each behavioral unit, where 0 = absense of the unit; 1 = occasional occurrence; 2 = constant presence during the interaction; and 3 = constant presence with very high intensity. Each interaction was assessed by 2–3 different observers, who were blind to the treatment applied, and the mean of the scores was considered as the final score for each behavioral unit.

Latencies (s) to the first approach and to the first bite (biting is the most important aggressive unit (29)), were also measured. Additionally another behavioral category, "relative spatial position" defined as the percentage of time that the fish spent at a distance of less than 2 cm during the interaction, was included. Three days after the injection the behavioral experimental sequence was repeated and recorded for each pair, in order to evaluate the reversibility of the effects induced by different treatments, and to rule out the incidence of habituation (36) on aggressive behaviors during interactions.

EVALUATION OF SPONTANEOUS SWIMMING ACTIVITY. A different group of animals was used in these experiments. Fish treated with either saline or MDMA were individually introduced into a tank 1 hr after injection. After 1 min of acclimatization, 4 min of spontaneous swimming activity were recorded on videotape. The percentages of time spent in motion and at rest were calculated for each experimental group.

Drugs

MDMA hydrochloride was synthesized and kindly provided by Dr. Bruce K. Cassels of the Universidad de Chile. The drug was dissolved in saline and doses of 1 and 5 mg/kg were used throughout the study.

Statistical Analysis

Mean interval of the EOD and standard deviation were calculated for each fish in control condition and after treatments. For the interaction experiments interval-phase plots (39) and cross-correlation histograms between the discharges of the two animals (isolated and interacting) were calculated. The EOD of the fish with the slower mean rate along the recording was used as time origin of the correlation. For each analysis time (100 msec and 1000 msec), the calibration of the ordinates are the same within each figure.

The chi-square test was applied to evaluate the significance of the difference in the percentages of JAR and non-JAR type histograms (see Results) in control and treated groups.

Differences in mean interval and NR amplitude between saline and MDMA treatments were evaluated with Student's *t*-test.

Comparison of the aggression measurements was performed using the Student's *t*-test in the case of continuous variables, such as latencies, and with nonparametric tests (Kruskal-Wallis analysis of variance followed by the Mann-Whitney *U*-test) in the case of the discontinuous variables such as the aggressive scores (rated 0 to 3) where normal distribution can not be assumed.

In all cases, the level of significance was set at $P < 0.05$, and indicated with asterisks.

RESULTS

EOD Recording of Individual Animals

EFFECTS OF MDMA ON RESTING DISCHARGE. The mean EOD interval (1 min recording) was slightly but significantly prolonged 1 hr after MDMA (1 and 5 mg/kg), in comparison with the values recorded in the same fish before injection (control group), and with the values of the group injected with saline (Fig. 1A). The standard deviation of the mean interval (1 min recording) was not significantly changed by MDMA (data not shown).

EFFECTS OF MDMA ON THE NR AMPLITUDE. The electric NR amplitude showed no significant changes due to MDMA injection. In the cases of visual and acoustic NR a tendency to increase was observed, but only in the visual sensory modality did this tendency reach a significant level (Fig. 1B).

EOD Recording During Two-Fish Interaction

In Fig. 2, the EOD interval of the faster fish (fish 1) is represented as a function of ongoing time (the upper horizontal plot). The EOD of the slower fish (fish 2) is represented as its latency with respect to the previous fish 1's EOD, plotted on the same time basis (the "climbing" plot). Thus, when fish 2 is the slower, the slope of the trace is positive ("climbing" plot), whereas a negative slope indicates that fish 2 is the faster ("descending" plots, not shown in the present article). This type of representation of EOD intervals has been used previously [*e.g.* (7,39)] and was chosen because it allows to recognize the occurrence of temporal coincidence between both signals (when the "climbing" plot intersects the horizontal plot).

It is important to point out that faster and slower fish often inverted this rate order, *i.e.*, the faster becoming the slower, but after the first 1–2 min of interaction a stable order was reached. Figure 2A corresponds to the animals in different tanks, which, therefore, were unable to sense each other's EOD. Figure 2B represents the same animals inter-

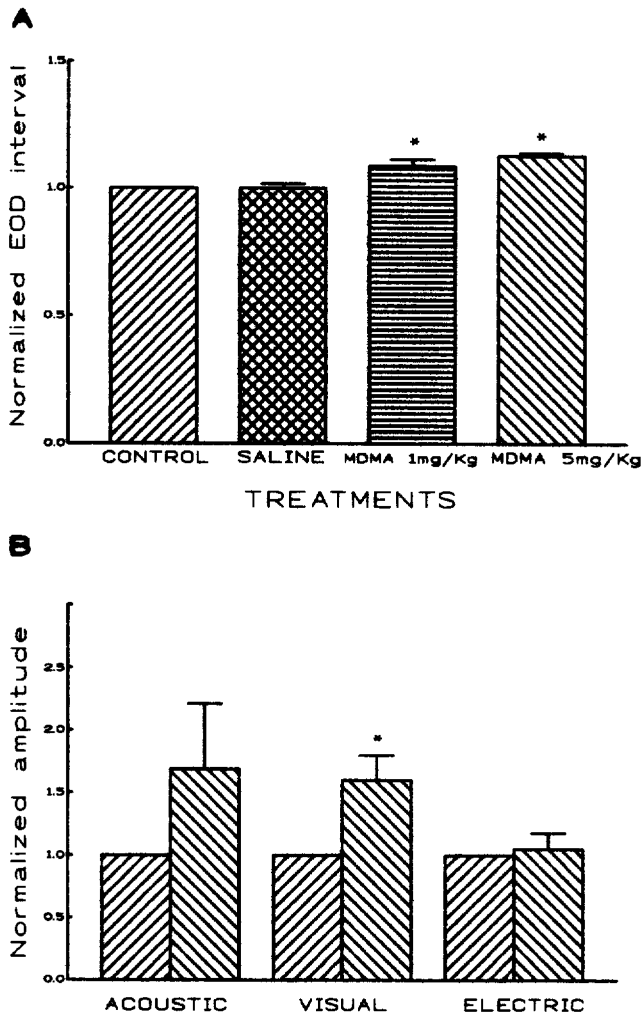

FIG. 1. Effects of MDMA on the EOD interval duration and on the novelty response amplitude. (A) Mean EOD interval (± SEM) of 1 min recording for non-treated, saline and MDMA (1 mg/kg and 5 mg/kg) groups ($N = 14$). The interval durations were normalized to the value of the control sample. *Significant difference with respect to saline-treated sample ($P < 0.05$). (B) Effects of MDMA (5 mg/kg) on the mean novelty response amplitude ± SEM, of acoustic, visual and electric sensory modalities ($N = 6$). The values for treated animals were normalized to the respective saline-treated controls. *Significant difference ($P < 0.05$).

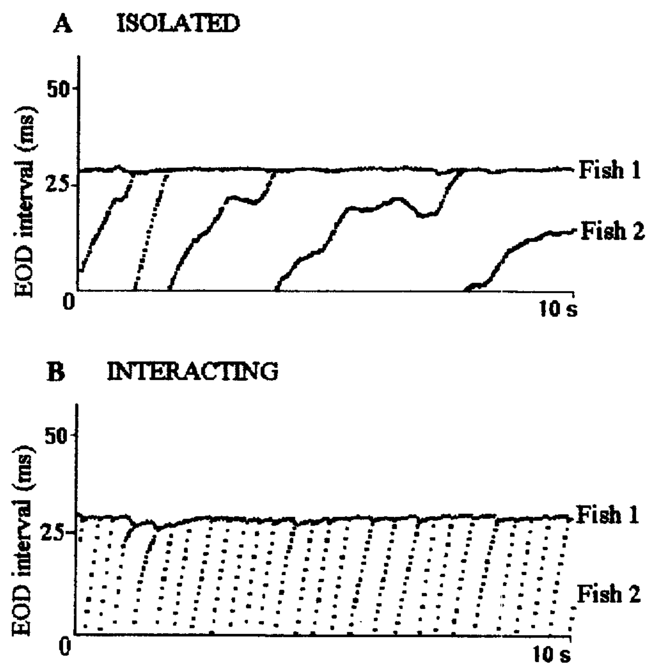

FIG. 2. Interval-phase plot of a fish couple in control condition. The EOD interval of fish 1 (faster) is shown as a function of ongoing time (horizontal plot), and the discharge of fish 2 (slower) is represented as its phase relative to the discharge of fish 1 plotted on the same time basis ("climbing" plot).

acting in the same tank. As can be seen in Fig. 2B, when an EODs temporal coincidence is close to occur the faster fish makes a rapid and brief frequency increase, which has the effect of avoiding the coincidence or shortening its duration. When the animals were isolated these increases were not observed (Fig. 2A). These frequency increases, which were found in about 50% of the cases, maintained the same magnitude during 5 min recordings, indicating that habituation did not occur. In most cases the slower fish maintained a stable rate of discharge with no decreases or increases.

This phenomenon could also be accounted for in the cross-correlation histogram of the EODs. Before presenting the results obtained using this type of analysis, we provide a general classification of the types of histograms observed.

CLASSIFICATION OF CROSS-CORRELATION HISTOGRAMS. The cross-correlations histograms were classified into two types. (a) JAR periodic histograms and (b) Non-JAR histograms. Within the second type we also distinguish two subtypes: non-JAR flat and non-JAR periodical.

Waving histograms with peak amplitudes decreasing from the origin to positive and negative values of time, were defined as type 1 (JAR periodical type). In these cases a minimum of the oscillation always lies at the origin of the cross-correlation histogram (*e.g.*, Figs 3B, and 4D). Type 2a corresponds to flat or almost flat correlations (*e.g.*, Fig. 4B). In type 2b, oscillations can be distinguished from basal noise. However, in these cases the peaks did not decay (or increase) from their point of origin, and the oscillations were more irregular than in type 1 (*e.g.*, Fig. 3A). In addition at the origin of the histogram either a maximum, a zero or a minimum point of the oscillation could be observed.

JAR IN THE CROSS-CORRELATION HISTOGRAM. When the two animals were isolated, the cross-correlation was of type 2. Generally, flat correlations (subtype 2a) were obtained, except in the cases in which the EOD rates of the two fish were very close, where 2b subtype histograms appeared.

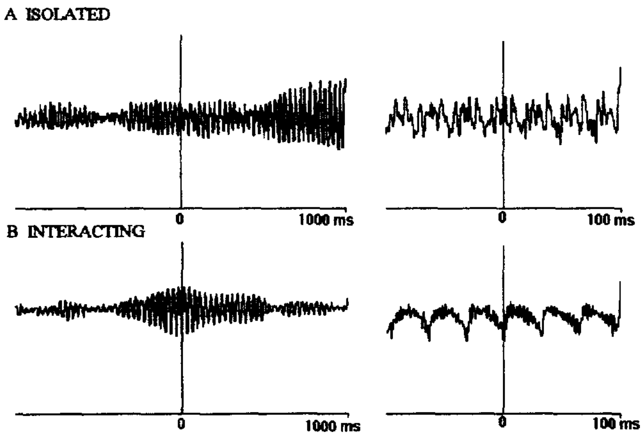

FIG. 3. JAR in the cross-correlation histogram. Cross-correlation histograms (5 min recording) of isolated (A) and interacting (B) condition in the same fish couple as in Fig. 2. The EOD of the fish with the slower mean rate along the recording (fish 2) was used as time origin of the correlation. The value of the time bin of the cross-correlation histograms is 4 msec for the 1000-msec analysis time (left column), and 0.4 msec for the 100-msec analysis time (right column). In (B) the period of the oscillation is equal to the mean interval of fish 2, which is 31.66 msec.

When the animals were placed together, JAR periodic oscillations (type 1) were found in about 50% of the cases (the remainder of the cases showing type 2 histograms).

In Fig. 3, cross correlation histograms of the same couple shown in Fig. 2 are represented. As can be seen, when these animals were separated the histogram was of subtype 2b (Fig. 3A). For conditions in which both fish are together, a type 1 histogram can be recognized (Fig. 3B). The time of this oscillation (Fig. 3B, right column) proved to be equal to the mean interval of the fish taken as time origin (see methods). As can be seen the minimum of the periodic oscillation lies at the origin of the cross correlation histogram (Fig. 3b right column), thus indicating a low probability of coincidence.

The appearance of the JAR-type periodic oscillations (type 1) correlates positively with the appearance of the frequency increases near the coincidences observed in the interval-phase plot. When no increases were observed in interacting fish, type 2 cross-correlations were obtained (*e.g.*, Figs. 4A and B).

EFFECTS OF PHARMACOLOGICAL TREATMENT. Figure 4 shows one case of an interacting couple with a flat correlation histogram (type 2a) and no fast frequency increases in the interval-phase plot (Figs. 4A and B); 1h after MDMA (1 mg/kg) injection the correlation became JAR periodical (type 1) and the interval-phase plot revealed fast increases (Figs. 4C and D).

As in the control group, saline-treated animals exhibited a type 1 (JAR) rhythmicity in about 50% of the cases, when placed together. In MDMA-treated couples, a significant

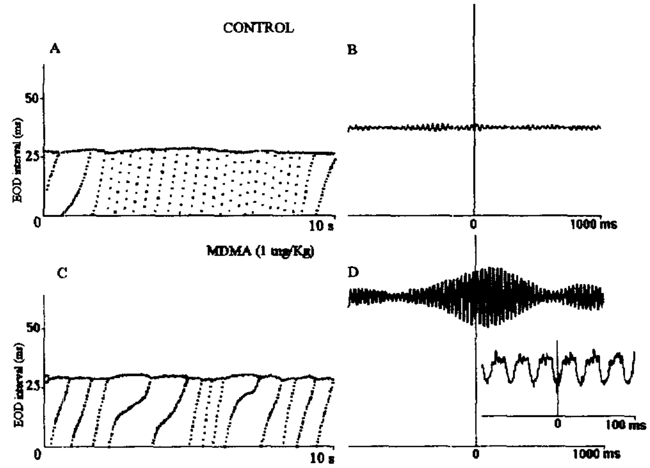

FIG. 4. MDMA may induce JAR. Interval-phase plot (A) and cross-correlation histograms (B) of a saline-treated interacting couple. (C) and (D): Same plots of the same couple interacting after MDMA (1 mg/kg). The value of the time bin of the cross-correlation histograms (5 min recording) is 4 msec for the 1000-msec analysis time, and 0.4 msec for the 100-msec analysis time shown in the inset. The mean interval of fish 2 is 30.81 msec, which is equal to the period of the oscillation shown in the inset of 5D. The frequency difference between both fish is 2.6 Hz in (A) and 1.4 Hz in (C).

increase in the frequency of appearance of this type of correlation was found (71% for 1 mg/kg and 90% for 5 mg/kg, see Fig. 5).

Besides the increase of probability of JAR type of EOD interaction, MDMA induced larger rate increases in the interval-phase plot (Fig. 6) in couples showing JAR in control conditions.

The EOD frequency difference (Hz) between both fish

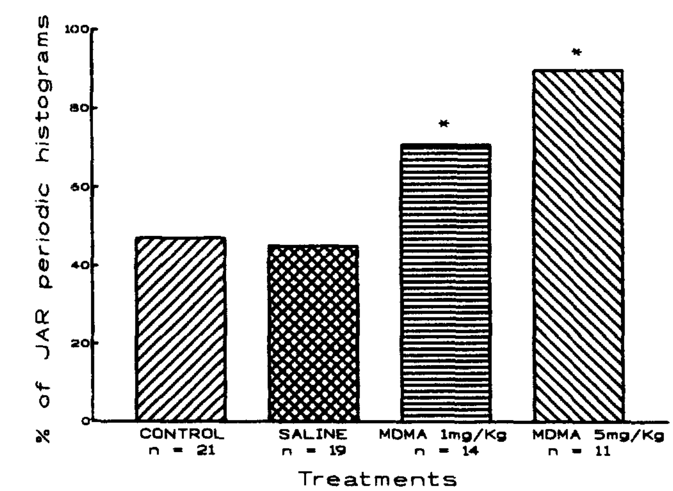

FIG. 5. MDMA increases the probability of JAR-type interactions. Percentage of JAR-type periodic cross-correlation histograms of couples under different treatments. *Significant difference with respect to saline-treated sample ($P < 0.05$).

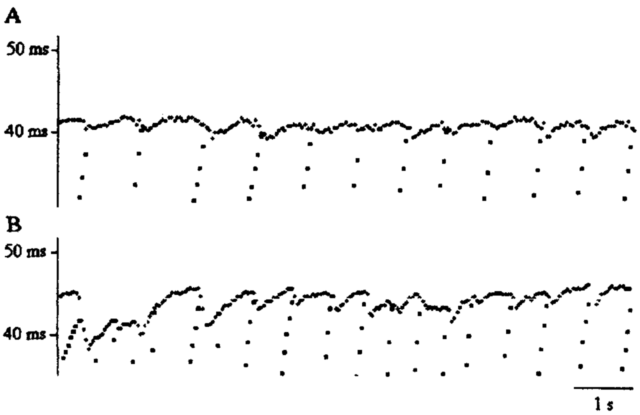

FIG. 6. MDMA may increase the amplitude of JAR. Interval-phase plot of a fish couple in control conditions (A), and after MDMA (5 mg/kg, B). Plots are presented in a larger scale (relative to Figs 2 and 4) in order to facilitate the comparison of JAR (minor values of slower fish's phases are truncated). The frequency difference between both fish is 12 Hz in (A) and 17 Hz in (B).

was smaller in interacting MDMA-treated couples than in the saline treated group, although these differences were not significant (data not shown). On the other hand, in the particular cases of couples that showed JAR after MDMA, and this response was absent in control conditions (*e.g.*, Fig. 4), the frequency difference in the treated group (1.41 ± 0.21 Hz, MDMA 5 mg/kg) was significantly smaller compared to the previous control sample (2.27 ± 0.22 Hz; $N = 7$).

Behavioral Assays

EVALUATION OF AGGRESSIVE BEHAVIOR. Tables 1 and 2 summarize the results obtained in the behavioral test.

Fish couples in the first interaction (control), *i.e.*, without drug or saline treatments, displayed in every case a clearly aggressive behavior, as expressed by the scores of the aggression units considered.

During the second interaction, 3 days later saline-vehicle–treated couples displayed a behavior that scored similarly to the previous ones. In the case of MDMA-treated couples, a significant and dose-dependent decrease was observed in most units (Table 1). The agonistic behavior was replaced by a clearly different pattern of interaction. In fact, some MDMA-treated couples showed a striking tendency to swim together in a parallel head-to-head position without any aggressive display. During this behavior, both fish were observed to swim from the water surface to the bottom of the tank without any "struggle" for spatial position. In some cases the animals tended to remain at rest in a head-to-head position. Furthermore, the latency to the first bite was clearly prolonged after MDMA treatments (Table 2). The latency to the first approach showed a tendency to increase in MDMA-treated groups, but it did not reach a significant level due to the high variability observed in these samples. The ratio of time spent together ("relative spatial position" category) during the interaction did not show any differences between treatments (Table 2).

In the third interaction, 3 days after treatments, aggressive behavior had recovered control level (no significant differences were observed between pre- and posttreatment interactions). This indicates that, under the present experimental conditions, the decrease of agonistic scores observed during the second interaction was not due to habituation phenomenon.

In the case of the latency to the first approach, MDMA-posttreated groups showed mean values clearly lower than the MDMA-treated samples and to the saline posttreated group. In spite of the high variability of these samples, these differences are very close to reach the significant level and may indicate some rebound effect after MDMA treatment. Nevertheless, these changes were not observed in the other aggressive measurements considered.

EVALUATION OF SPONTANEOUS SWIMMING ACTIVITY. Figure 7 shows that spontaneous swimming was significantly diminished in animals treated with 5 mg/kg MDMA, as compared with the saline-vehicle–treated population. The group injected with 1 mg/kg did not show any significant difference in comparison with control groups.

TABLE 1. Comparative effects of treatments on aggression. Aggression scores for bite, serpentining (serp.), jaw locks, and approach behavioral units. Pretreated, treated, and posttreated conditions are shown for saline, MDMA 1 mg/kg and 5 mg/kg groups ($N = 10$ fish couples)

Aggressive unit	Saline			MDMA 1 mg/kg			MDMA 5 mg/kg		
	Previous	Treated	Posttreated	Previous	Treated	Posttreated	Previous	Treated	Posttreated
Bite	1.7 ± 0.3	2.1 ± 0.2	1.9 ± 0.3	2.1 ± 0.2	0.6 ± 0.2*	2.2 ± 0.2	2.2 ± 0.2	0.1 ± 0.1*	2.1 ± 0.4
Serp.	2.1 ± 0.3	2.2 ± 0.3	1.7 ± 0.4	1.8 ± 0.3	0.7 ± 0.3*	1.4 ± 0.2	2.2 ± 0.2	0.5 ± 0.2*	1.9 ± 0.4
Jaws lock	0.5 ± 0.3	1.0 ± 0.5	0.8 ± 0.6	1.1 ± 0.6	0.2 ± 0.2	0.7 ± 0.5	1.0 ± 0.3	0.0 ± 0.0*	0.3 ± 0.2
Approach	2.4 ± 0.3	2.3 ± 0.4	2.2 ± 0.2	2.4 ± 0.2	1.9 ± 0.3	2.2 ± 0.2	2.7 ± 0.2	0.5 ± 0.4*	2.6 ± 0.3

*Significant difference with respect to either the saline-treated sample (MDMA groups) or to the previous value of each group (posttreated condition) ($p < 0.05$).

TABLE 2. Bite and approach latencies (s) and percentage of time together in previous, treated and posttreated conditions for saline, MDMA 1 mg/kg, and 5 mg/kg groups (N = 10 fish couples)

	Saline			MDMA 1 mg/kg			MDMA 5 mg/kg		
	Previous	Treated	Posttreated	Previous	Treated	Posttreated	Previous	Treated	Posttreated
Bite latency (s)	85.7 ± 38.9	69.2 ± 26.5	83.2 ± 38.3	51.9 ± 17.7	237.6 ± 25.5*	46.5 ± 25.2	59.0 ± 25.0	275 ± 25.0*	66.0 ± 40.0
Approach latency (s)	24.3 ± 13.6	28.3 ± 16.3	61.4 ± 40.3	24.4 ± 14.7	61.6 ± 38.2	4.8 ± 1.3	12.8 ± 4.3	76.3 ± 38.0	3.1 ± 1.1
% Time together	89 ± 4.0	85 ± 6.0	82 ± 8.0	91 ± 5.0	78 ± 12.1	79 ± 10.0	93 ± 20.0	73 ± 13.4	77 ± 13.2

*Significant difference with respect to either the saline-treated sample (MDMA groups) or to the previous value of each group (posttreated condition) ($P < 0.05$).

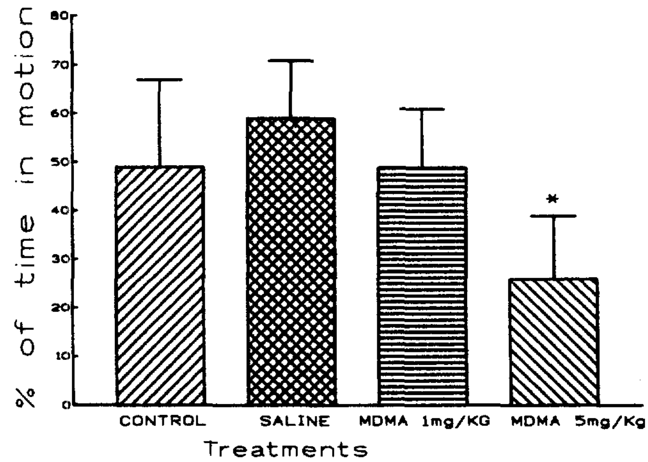

FIG. 7. High doses of MDMA may decrease spontaneous motor activity. Percentage of spontaneous motion time of single animals under different treatments (N = 10). *Significant difference with respect to saline-treated sample ($P < 0.05$).

DISCUSSION

MDMA Effects on the Mean EOD Interval and NR Amplitude

Pharmacological treatments that enhance serotonergic (5-HT) neurotransmission have been reported to induce in *Gymnotus carapo* an increase (around 80%) of the mean EOD interval duration. Additionally the appearance of spontaneous frequency bursts that increase the standard deviation of the discharge nearly 4-fold was observed. NR amplitude to electric, visual, acoustic, and mechanical stimuli was also increased several-fold after these treatments. These effects have been associated with the activation of 5-$HT_{2A/2C}$ receptor subtypes (8).

MDMA has been reported to induce the release of 5-HT 0.5–3 hr after injection in rats, leading thereafter to a prolonged decrease in brain indole levels (30,33). There is evidence that this drug may also release dopamine [(31,40); for a review see (24)].

In this study, MDMA (5 mg/kg) consistently lengthened the EOD mean interval, by about 13%, which is clearly less than that observed with the treatments mentioned above. No spontaneous frequency bursts were observed and the standard deviation was unchanged after MDMA-treatment. NR to visual stimuli showed a significant increase with MDMA (about 50%), although this effect was less than that observed with 5-HT precursor and agonists (8). NR amplitudes were not significantly affected in the cases of electric and acoustic modalities.

Some of these changes, such as the duration of the EOD interval and the increase in visual NR amplitude, might be due to increased 5-HT release. The absence of significant effects on the standard deviation of the EOD interval, which is a reflection of the regularity of the discharge, and on the electric and acoustic NR amplitudes might be explained both by the interactions of MDMA with neurotransmitters other than 5-HT, and/or by a different effect of MDMA upon serotonergic transmission than those of the treatments mentioned above.

Interaction of Fish Pairs

JAR IN *GYMNOTUS CARAPO*. The results reported here show that when two *Gymnotus carapo* are placed together, brief frequency increases in the EOD rate of the faster fish of the pair are observed when an EOD coincidence is close to occur. This behavior showed no habituation during at least 5 min. From the analysis of the cross-correlation histograms of interacting fish EODs, it may be seen that these brief increases have the effect of minimizing the likelihood of coincidence between EODs. Thus, this behavior is a type of JAR and corresponds to that previously described in curarized animals (1,2,18). Westby (38) stimulating *Gymnotus carapo* with electric pulses phase-locked to the EOD, reported that a stimulus occurring approximately in the last 5 msec of the EOD cycle induces an oscillation in the EOD frequency which the author identified as jamming avoidance response (JAR). Frequency increases reported here (Fig. 2B) also occur when the slower fish's EOD is in the last few milliseconds of the faster fish's phase. In experiments with artificial pulses which scan the EOD cycle of *Gymnotus carapo* at a controlled velocity (*i.e.*, the experimenter can control the magnitude and sign of the frequency difference between the fish EOD and the artificial pulse), Lorenzo *et al.* (personal communication) have observed that transient frequency increases were the most common reaction to electric pulses occurring in this "phase window," and that left to right scanning pulses were more efficient than right-left scanning or phase locked pulses. Thus, the increases recorded in interacting animals may represent the same behavioral reaction observed under phase-locked stimulation, but acting in a predominantly free running condition.

There has been reported that the hierarchical status in *Gymnotus carapo* is correlated with the EOD rate, the faster fish being the dominant of the group (37). In this context, the observation that after a few minutes of interaction a stable order of EOD rates was reached suggests that at this time hierarchical relationships were established.

EFFECTS OF MDMA. After MDMA injection, the probability of occurrence of JAR was significantly enhanced. In most cases, both amplitude and duration of these frequency increases were also enhanced. This increase in a social behavior aimed at preserving electrolocation (JAR) was associated with a nonaggressive and even "friendly" interaction in the behavioral tests. Both behavioral changes could have some common features with the changes in mood reported with MDMA in healthy human volunteers.

Lorenzo *et al.* (personal communication) have observed that 0.5–1.5 Hz are the most efficient frequency differences (Hz) between a fish and an electric stimulus mimicking an EOD in order to induce JAR. Thus, the increase in JAR probability induced by MDMA may be due to the smaller frequency differences observed in after treatment (*e.g.*, Fig. 4). Nevertheless the increase in amplitude of JAR observed some MDMA treated couples without a change in frequency difference (*e.g.*, Fig. 6) may imply the involvement of other mechanisms.

Although the spontaneous motor activity was decreased by 30% after 5 mg/kg of MDMA in comparison with saline-treated animals, effects on aggressive display are not likely to be due to general sedation, because there was no significant variation in the latency to the first approach nor in the fraction of time spent together during the interaction. In fact, the aggressive display in MDMA-injected animals was replaced by a distinct pattern of interaction but not by a non-interactive behavioral pattern. Furthermore, 1 mg/kg of MDMA had no significant effect on spontaneous motor activity, whereas aggression was greatly diminished. In addition, some behaviors, such as visual NR and JAR, were specifically enhanced by the treatment, and the ability of the animals to avoid being caught with a net was clearly not impaired, thus excluding the possibility that the effects observed on aggression could be due to general CNS depression.

The decrease in spontaneous motor activity in 5 mg/kg MDMA-treated *Gymnotus carapo* contrasts with the effect of this drug in rats, where motor activity has been reported to increase after the same dose (13,32).

The activation of 5-$HT_{2A/2C}$ receptor subtypes, a common feature of psychotomimetic drugs, has been associated with an increase in the intensity of sensory perception and the appearance of psychedelic phenomena in human (11,12), and with the increase of NR amplitude of all sensory modalities in *Gymnotus Carapo* (8). According to the human reports, MDMA [which has low affinity for 5-$HT_{2A/2C}$ receptors (22,24)], induces different sensory changes, while an increase in empathy seems to be the most remarkable characteristic. The results shown above indicate that in *Gymnotus carapo* the effects of MDMA upon the pattern of interaction are very pronounced, while the effects on NR amplitude of different sensory modalities are not as striking as in the case of 5-$HT_{2A/2C}$ agonist treatment. This seems to be in agreement with observations that suggest that psychedelics and entactogens are different classes of drugs (27).

Concerning the mechanisms underlying the effects of MDMA reported here, little can be said at present. It has been reported that a massive release of 5-HT takes place 0.5–3 hr after acute MDMA treatment in rats. This effect leads to a pronounced decrease of endogenous 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels, which return to normal within 24 hr after injection (24). This 5-HT release is likely to be involved in the neurochemical mechanisms of the effects presented here. In fact, monoamines have been associated with the modulation of aggressive signals in the Gymnotiform fish *Apteronotus leptorhynchus* (23); in this species the distribution pattern of central serotonergic pathways is very similar to that found across vertebrate taxa, including amphibians, reptiles, and mammals (19). Furthermore, preliminary results of our group have shown that MDMA inhibits in a dose dependent manner the territorial and maternal aggressive behavior in rats (unpublished). In this context, the similarity of MDMA effects on the aggressive behavior in fish and humans may imply that these effects are caused by or at least linked to a very conservative mechanism.

However, MDMA is a nonspecific drug and interactions with neurotransmitter systems other than serotonergic should be considered. Further studies with more specific serotonergic agents are needed in order to asses the specific role of 5-HT in the JAR and aggressive behavior in *Gymnotus carapo*.

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