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Actions of MDMA at glutamatergic neuromuscular junctions

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") compels mammalian serotonergic neurons to release serotonin (5-HT). In this study, MDMA altered synaptic transmission presynaptically by enhancing quantal release in two model glutamatergic synapses—the neuromuscular junction (NMJ) of the crayfish opener muscle, which is enhanced by exogenous 5-HT application, and the NMJ of a larval body wall muscle in *Drosophila melanogaster*, which is insensitive to exogenous 5-HT application. At the crayfish NMJ, MDMA mimicked the actions of 5-HT but only at a substantially higher concentration. At the *Drosophila* NMJ, MDMA altered synaptic transmission but not through a 5-HT receptor.

Using simple invertebrate preparations, we have demonstrated an additional non-serotonergic mechanism of MDMA activity that has not yet been addressed in vertebrate systems and that may play an important role in understanding the mechanism of action for a commonly abused drug.

Keywords: MDMA; Serotonin; Neuromodulation; Synapse; Crayfish; Presynaptic; *Drosophila*

1. Introduction

MDMA evokes release of serotonin (5-HT), dopamine (DA), and norepinephrine (NE) from mammalian neurons (Geyer and Callaway, 1994; Green et al., 1995; Sprague et al., 1998). Previous studies have suggested that MDMA has direct agonist effects at 5-HT_{2A} and 5-HT_{2C} receptors (Nash et al., 1994). MDMA-induced release of 5-HT depletes the 5-HT stores. Evidence also suggests that reversal of the 5-HT transporter may be responsible for some of the increase in synaptic 5-HT concentration (Rudnick and Wall, 1992). The overall increase of extracellular 5-HT levels modulates the activity of 5-HT-sensitive synapses within various central circuits.

In adult mammalian models, damage to central serotonergic neurons after exposure to doses of MDMA, similar to human recreational doses per body weight causes depletion of 5-HT, and 5-HT transporters cannot be detected for months (Stone et al., 1986; Battaglia et al., 1987; Schmidt, 1987; Ricaurte et al., 1988, 1992). In human studies, MDMA users have lower levels of 5-HT in the cerebrospinal fluid, while metabolites for dopamine or norepinephrine are not reduced (McCann et al., 1999). Despite these adverse effects, MDMA may have some therapeutic value in humans, as it seems to be effective in relieving short-term, post-traumatic stress (Jansen, 1999) and reverses the effects of haloperidol-induced Parkinsonism in the rat model (Schmidt et al., 2002).

Although the actions of MDMA have been studied extensively (Holland, 2001), no proposed mechanism of the action of MDMA completely explains the varied responses observed in the variety of mammalian preparations examined. The complex nature of the differences in acute and chronic effects of MDMA makes the mechanisms of these actions incredibly difficult to study in complex systems. For example, MDMA-induced depletion of 5-HT causes chronic up-regulation of 5-HT_{2A} receptors in the occipital cortex of former MDMA users (Reneman et al., 2002). MDMA responses may also alter the regulation of the secondary cellular cascades in conjunction with receptor up-regulation, thus altering cellular processes that utilize the same pathways.

Abbreviations: 5-HT, 5-hydroxytryptamine; DA, dopamine; EPSP, excitatory post-synaptic potential; fEPSP, field excitatory post-synaptic potential; fmEPSP, field miniature excitatory post-synaptic potential; *m*, mean quantal content; MDMA, 3,4-methylenedioxymethamphetamine; NE, norepinephrine; NMJ, neuromuscular junction; STF, short-term facilitation

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Some of the mechanisms of action described for MDMA can be addressed in simple model systems using isolated synaptic preparations in order to reduce a number of complex variables within whole animals. Proposed mechanisms developed through investigations of simple invertebrate systems can then be extrapolated to complex mammalian systems. While some actions may vary between invertebrate and vertebrate systems, the highly conserved nature of synaptic transmission bridges many species. We have noted that MDMA alters synaptic function in two model glutamatergic synapses: the crayfish opener muscle neuromuscular junction (NMJ), which is enhanced by exogenous 5-HT application, and the NMJ at a *Drosophila melanogaster* larval body wall muscle, which is insensitive to exogenous 5-HT application. The 5-HT-sensitive preparation responds primarily to 5-HT_{2A}-receptor agonists and antagonists commonly used in vertebrate studies (Tabor and Cooper, 2002). Since MDMA enhances synaptic transmission at both the 5-HT-sensitive crayfish NMJ and the 5-HT-insensitive *Drosophila* NMJ, we suggest that MDMA must be enhancing transmitter release by a non-serotonergic mechanism in addition to its serotonergic effects. As far as we are aware this is the first report of MDMA enhancing release at a glutamatergic synapse.

A preliminary report of these findings was presented in abstract form (Cooper et al., 2003).

2. Methods

The methods are similar to those described previously for the fly and crayfish (Sparks et al., 2003). In brief, the following procedures were followed:

2.1. Animals

Mid-sized crayfish (*Procambarus clarkii),* measuring 8–10 cm in body length and weighing 15–20 g, were obtained from Atchafalaya Biological Supply Co. (Raceland, LA). Animals were housed in an aquatic facility within the laboratory in individual tanks and were fed fish food pellets every 3 days. Only male crayfish in their intermolt stage were used.

The "wild-type" laboratory strain of *Drosophila melanogaster*, Canton S, was used in these studies. The eggs were allowed to hatch and develop at 25 °C with a 12 h:12 h dark–light cycle. The methods used to stage fly larvae have been described previously (Li et al., 2002). All animals were maintained in vials partially filled with a cornmeal–agar–dextrose–yeast medium. Larvae at the beginning of the "wandering" phase of the third instar were used in these experiments.

2.2. Dissection and physiology

The dissection and electrophysiological procedures used in this study have been described in detail (Cooper et al., 1995a; Cooper and Neckameyer, 1999; Dasari and Cooper, 2004). All experiments were performed at room temperature (17–19 °C).

2.3. Pharmacology

In the crayfish and *Drosophila* studies, 5-HT (Sigma Co., St. Louis, MO) or MDMA (supplied by NIH–NIDA) were applied exogenously. The concentrations used are reported in the results for each experimental paradigm.

2.4. Intracellular evoked excitatory post-synaptic potentials (EPSPs)

Intracellular muscle recordings were made with a 3 M KCl-containing microelectrode. Short-term facilitation (STF) was induced at the crayfish NMJ by applying a 40 Hz train of 10 pulses at 10 s intervals. The facilitation index for STF was determined by the amplitude of the 10th EPSP to the 5th within a train (Crider and Cooper, 1999, 2000).

2.5. Field excitatory postsynaptic potentials (fEPSPs)

At the crayfish and fly NMJ, synaptic potentials were also measured with focal macropatch electrodes to determine the effect of MDMA on presynaptic vesicular events. The varicosities on the living terminals at crayfish NMJs were visualized by the use of the vital fluorescent dye 4-Di-2-ASP (Molecular Probes) (Magrassi et al., 1987; Cooper et al., 1995a) and for the fly the terminals were viewed with Nomarski optics. Field excitatory postsynaptic potentials (fEPSPs) were recorded in conjunction with a 0.1× LU head stage and an Axoclamp 2A amplifier in a bridge mode configuration. The fEPSP synaptic potentials were obtained using the loose patch technique by lightly placing a 10–20 μm fire polished glass electrode directly over a spatially isolated varicosity along the nerve terminal that were viewed under a 40× water immersion lens (Nikon, NA 0.55). The macropatch electrode is lightly placed over the varicosity to form a loose seal (100–200 kΩ) which measures the potentials preferentially within the electrode lumen. The number of quantal events within the fEPSPs were recorded and analyzed to determine the mean quantal content (*m*) for the crayfish NMJ (Cooper et al., 1995b, 1996). Since the evoked release recorded at the *Drosophila* NMJ are multiquantal the average area of mfEPSPs and the average area of fEPSPs were used to determine mean quantal content (Cooper et al., 1996).

At the crayfish NMJ direct counts of the number of evoked quantal events and failures in evoked release were used as an index of altering synaptic function. If only one single event occurred after the spike, it was counted as one; when double events occurred, they were counted as two, etc. Quantal release over time was also monitored by examining the area of the evoked potential. Since peak amplitude in evoked events can vary due to latency jitter when multiple events

occur, we used a more reliable measure of area under the recorded trace for the events (Cooper et al., 1995b). The crayfish tonic nerve was stimulated at a rate of 1 or 2 Hz and for the *Drosophila* NMJ at 0.5 Hz in order not to facilitate the responses between trials.

2.6. Larval Drosophila neuromuscular junctions

The dissections were performed as described earlier (Cooper et al., 1995b; Sparks et al., 2003). The electrical recordings were obtained from the prominent longitudinal m6 muscle. Selective stimulation to the axons that give rise to the Ib and the Is terminals was produced by altering the stimulus intensity and duration while monitoring the EPSP amplitudes in m6. Since the EPSP amplitudes are reflective of the type of terminal responding this measure was used (Harrison and Cooper, 2003; Kurdyak et al., 1994). The physiological solution, HL3, is the same as previously described (Stewart et al., 1994). All experiments were performed at room temperature (19–21 °C).

3. Results

The innervation of the crayfish opener muscle consists of polyneuronal innervation of an inhibitor (GABAergic) and excitor (glutamatergic) motor neurons. The excitor can be selectively stimulated in a proximal location in a preceding leg segment (Dudel and Kuffler, 1961). The innervation is also multi-terminal with a motor nerve terminal branching on a given muscle fiber with intermediate varicosities (i.e., swellings) where the synaptic contacts reside (Fig. 1A). The innervation produces graded electrical responses on the muscle fibers without spiking. Thus, the muscle fiber is similar to a dendrite in a vertebrate brain in respect to electrical integration on the membrane. Intracellular recordings obtained from the muscle fibers record the excitatory postsynaptic potentials (EPSPs) on the whole muscle cell. Since a single evoked stimulus does not substantially depolarize the muscle fiber, a train of stimuli is provided to produce facilitated EPSPs (Fig. 1B). A 40 Hz train of 10 pulses gives rise to easily measured facilitated EPSPs (Crider and Cooper, 2000). The amplitude of the 5 and 10th EPSPs within the train are commonly measured for comparative purposes and for addressing the effects of pharmacological agents in this preparation (Southard et al., 2000; Cooper et al., 2001a; Tabor and Cooper, 2002; Sparks et al., 2003).

A focal macro patch electrode placed directly over the synaptic sites, contained within a varicosity, records evoked field excitatory postsynaptic potentials (fEPSPs) and field miniature excitatory postsynaptic potentials (fmEPSPs) from defined regions of a living terminal (Fig. 1C). In the recording shown, the evoked response occurs after the extracellular spike due to the action potential invasion of the terminal (Fig. 1D). The events that occur after the rapid evoked responses are deemed as spontaneous events. The

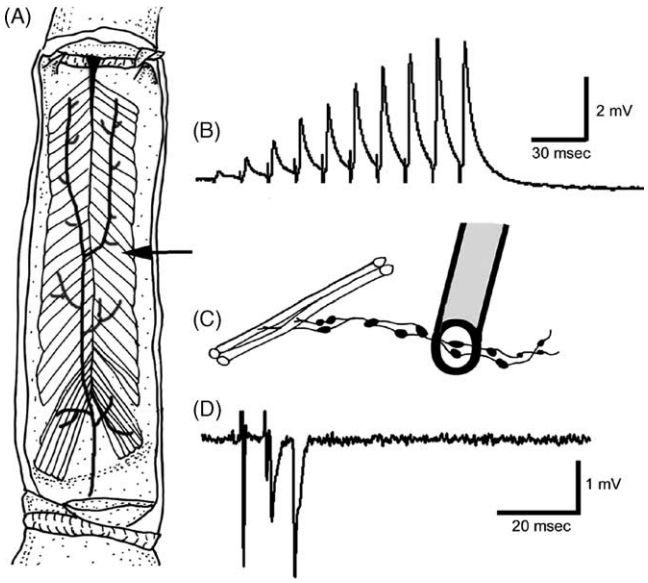

Fig. 1. The crayfish opener muscle of the first walking leg. The muscle is innervated polyneuronally by inhibitor (GABAergic) and excitor (glutamatergic) motor neurons. (A) Stimulation by a 40 Hz train of 10 pulses provided to the motor nerve gives rise to excitatory postsynaptic potentials (EPSPs) measured by intracellular recordings. (B) Visualization of the varicosities on the motor nerve terminals innervating the muscle provides an additional advantage of the preparation since a focal macro patch electrode is able to be directly placed over the synaptic sites. (C) Evoked field excitatory postsynaptic potentials (fEPSPs) and field miniature excitatory postsynaptic potentials (fmEPSPs) are recorded from defined regions of the living terminals. (D) Two evoked quantal events are shown in the depicted trace.

recordings are selective for the region in which the electrode lumen is placed (Cooper et al., 1995b). Currents residing outside the lumen are represented by an opposite direction in the field recordings. This technique allows one to monitor single vesicular events in order to determine if MDMA enhances the intracellular recorded EPSPs through an increase in presynaptic transmission, altered postsynaptic responsiveness to glutamate, or a combination of the two possibilities.

Application of MDMA (10 μM) to the crayfish muscle preparation resulted in a gradual increase in the amplitude of the intracellular obtained EPSPs (Fig. 2A). The facilitation index before and during exposure to MDMA did not significantly change (data not shown), which suggests that MDMA is not influencing the parameters associated with short-term facilitation. In comparison to 5-HT, high concentrations of MDMA are needed to obtain measurable effects on synaptic enhancement. An exposure to 100 nM 5-HT to the crayfish NMJ enhances the 10th EPSP amplitude within a 40 Hz train by approximately 200% (Fig. 2B; $P < 0.05$, non-parametric rank sum Wilcoxon test). Exposure to 10 μM 5-HT produced massive contractions upon stimulation as well as pronounced spontaneous transmission. In contrast, exposure to 10 μM MDMA alone produced approximately a 40% increase and for a cocktail of 10 μM MDMA and

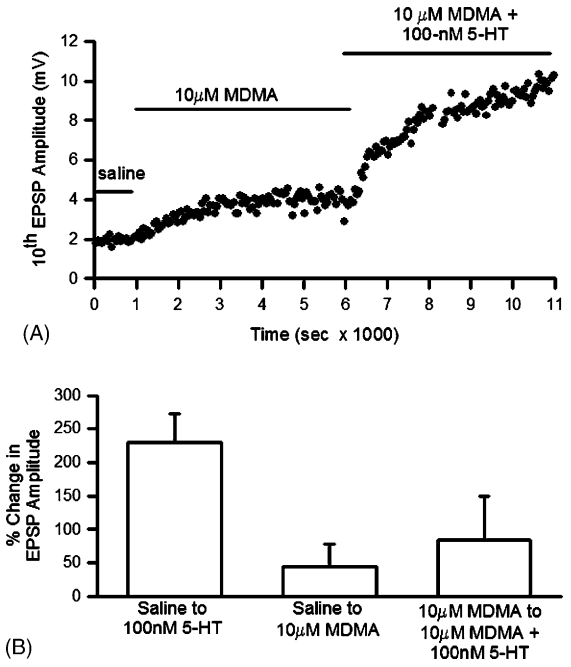

Fig. 2. Effect of MDMA on EPSP amplitude recorded from the opener muscle of crayfish. MDMA (10 μM) at the crayfish NMJ caused a gradual increase in the amplitude of the intracellular obtained EPSPs. (A) A 100 nM exposure of 5-HT to the crayfish NMJ enhances the 10th EPSP amplitude within a 40 Hz train by approximately 200%. (B $P < 0.05$, non-parametric rank sum Wilcoxon test). MDMA exposure at 10 μM produces a 45% increase of the 10th EPSP amplitude (A & B $P < 0.05$, $n = 5$ preparations, non-parametric rank sum Wilcoxon test). Exposure of the NMJ preparation to MDMA (10 μM) combined with 5-HT (100 nM) after the preparation had already been exposed to MDMA (10 μM) produces an 84% enhancement of the EPSP amplitude (B).

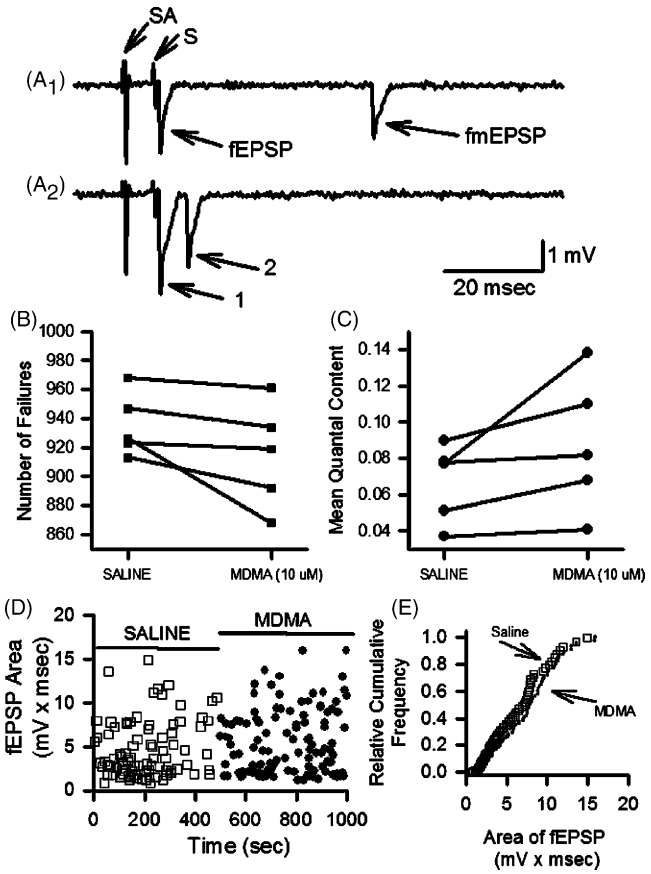

Fig. 3. Quantal recordings from defined regions of the excitatory motor nerve terminal on the opener muscle. Quantal releases were monitored by directly counting the number of quantal events in the field excitatory postsynaptic potentials (fEPSPs) obtained in focal macropatch recordings from defined regions of the motor nerve terminals. The stimulus artifact (SA), the nerve terminal spike (S), and a single evoked response are readily measured (A1). Two evoked events are shown in the trace (A2). The number of failures in evoking a response decreased upon exposure to MDMA (B) $P < 0.05$, $n = 5$ preparations, non-parametric rank sum Wilcoxon test. Mean quantal content for 1000 stimulus trials prior to and during exposure to MDMA (10 μM) increased upon exposure to MDMA (C) $P < 0.05$, non-parametric rank sum Wilcoxon test. Measures of the area under the trace for evoked single fEPSPs before and during exposure showed no differences in the area distributions (D). Upon normalization for the number of increased events during exposure to MDMA, the relative cumulative frequency plot in the areas of the single fEPSPs showed no substantial shifts in the distributions, implying that MDMA has no effect on postsynaptic receptivity of released glutamate (E).

100 nM 5-HT an increase of 84% of the 10th EPSP amplitude occurs (Fig. 2B; $P < 0.05$, non-parametric rank sum Wilcoxon test). Control runs were made for 1 h and 30 min with and exchange of saline after 30 min. No differences in the amplitude of the EPSPs occurred in these conditions for exposure to saline alone ($n = 5$).

We monitored the field potentials (fEPSPs) and quantified effects on presynaptic release by counting the number of failures in evoking a response as well as the number of evoked events. Failure to evoke an event is noted by the absence of an fEPSP within a 20 ms time frame after the stimulus artifact and nerve terminal spike (i.e., an extracellular recorded action potential of the nerve terminal). However, miniature (spontaneous) events (fmEPSPs) can be recorded outside of the 20 ms time frame following nerve terminal depolarization (Fig. $3A_1$). The 20 ms period of time was deemed as the window of time that evoked events occur since an ensemble average of evoked events only produces a deflection within this time frame; however it is still an arbitrary period but useful as an comparative index. When single or multiple evoked events occur they are directly counted (Fig. $3A_2$). Mean quantal content may be underestimated due to multiple firings within a single evoked response when the latency of release is short among multiple events as small evoked events maybe masked by larger evoked events (Cooper et al., 1995b). However, the decrease in the number of failures reliably demonstrates an increase in the number of evoked events during exposure to MDMA (Fig. 3B; $P < 0.05$, non-parametric rank sum Wilcoxon test), suggesting that MDMA enhanced synaptic transmission presynaptically. The number of evoked events were counted and indexed as a mean quantal content for 1000 stimulus trail prior to and during exposure to 10 μM MDMA after incubation of the preparation for 5 min (Fig. 3C; $P < 0.05$, non-parametric rank sum Wilcoxon test). The masking of

Table 1
The number of failures (0), evoked single (1) as well as multiple events (2 or greater) are shown for five preparations

	Obs	
	Saline (0–1000)	MDMA (0–1000)
Prep 1		
0	923	919
1	76	80
2	1	1
m	0.078	0.082
Prep 2		
0	926	868
1	72	126
2	1	6
3	1	0
m	0.077	0.138
Prep 3		
0	968	961
1	29	37
2	1	2
3	1	0
4	1	0
m	0.037	0.041
Prep 4		
0	947	934
1	49	64
2	2	2
m	0.051	0.068
Prep 5		
0	913	892
1	84	106
2	3	2
m	0.09	0.11

These counts are from the fEPSPs traces obtained by focal macro-patch recordings. In preparation 4 two sweeps were discarded due to a failure in evoking a nerve terminal spike, thus 998 stimulus trials were used.

small evoked events did not seem to be a major concern in these preparations, since a low stimulation frequency was used to reduce multiple firing.

We observed no significant difference in the area under the trace of single evoked fEPSPs or spontaneous events. Measures of the area under the trace for evoked single fEPSPs before and during exposure showed no differences in their distributions (Fig. 3D). Upon normalization for the number of increased events during exposure to MDMA, a plot of the relative cumulative frequency in the areas of the single fEPSPs showed no substantial shift in distribution (Fig. 3E).

The number of failures and evoked events are shown in Table 1 for the five preparations utilized. Since multiple analysis protocols are utilized in the synaptic transmission field (Del Castillo and Katz, 1954; Cooper et al., 1995b, 1996; Viele et al., 2003) to estimate the number of release sites and the probability of release, we provide the original values for further computational assessment by other investigators using their analysis protocol of choice.

The innervation and synaptic properties of the Is and Ib motor nerve terminals that innervate muscle 6 of the third instar *Drosophila* larva have been thoroughly studied (Atwood et al., 1993; Kurdyak et al., 1994; Li et al., 2002). The Ib and Is terminals have different morphology and physiology, though they can be recruited separately or in unison. The terminals of the Is axon contain small varicosities along its length and give rise to large EPSPs in the muscle, while the Ib axon has large varicosities and produces smaller EPSPs (Atwood et al., 1993; Kurdyak et al., 1994; Stewart et al., 1994) (Fig. 4A and B). The induced depolarization in these muscles, like the crayfish preparation, are graded, non-spiking and glutamatergic. MDMA (10 μM) produced a slight enhancement of the EPSP amplitude in each preparation examined (Fig. 4C, $P < 0.05$, non-parametric rank sum Wilcoxon test). The EPSP amplitude gradually decayed to near-baseline values after a few minutes. These preparations are less stable than the crayfish NMJs. There was variation among the preparations to the onset and peak of the effect. In five preparations the mean time to the onset of the effect was 154 s (±43 s, S.E.M.) and the mean time to the peak of the effect was 208 s (±41 s, S.E.M.). The time to onset is defined as the time from application of MDMA to the time the EPSPs started to increase in size and the time to peak as from the time of application to the maximum response in the EPSP amplitudes. The NMJs of m6 are insensitive to 5-HT application; an exposure to 10 μM 5-HT produced no significant effect on the amplitude of the composite Ib and Is EPSP as compared to the run down of the EPSP amplitudes over time. Saline controls ($n = 5$) were conducted for the same period of time in order to measure the degree of run down in the responses at the *Drosophila* NMJs as compared to exposure to 5-HT or MDMA (Fig. 1D).

As with the crayfish NMJ, we next addressed if the changes observed in the EPSPs are due to presynaptic effects by use of focal macropatch recordings over Is varicosities. The distal ends of the Is terminals were used to help insure isolation from the Ib terminals. Although the changes are small there is a consistent increase in the area of the fEPSPs after exposure to MDMA without any measurable differences in characteristics of spontaneous events. A typical record of an fEPSP with evoked and a spontaneous event (fmEPSP) is shown (Fig. 4E) and the area for the fEPSP before and during MDMA exposure is illustrated (Fig. 4F). The percent change in the area of the fEPSP varied but produced an increase (6.2, 8.1 and 14%; $n = 3$). The percent difference was obtained during the peak response from MDMA exposure as compared to saline exposure. The effect varied in time to onset and time to peak responses just as for the intracellular responses. The mean quantal content of the three preparations before (3.633, 5.331 and 3.669) and during exposure to MDMA (3.928, 5.662 and 4.197) within the 400–800 s, bracketing the peak change induced by MDMA, revealed the same percent increase as the change in the area of the evoked responses. The mean increase is 9.57% for the mean quantal content.

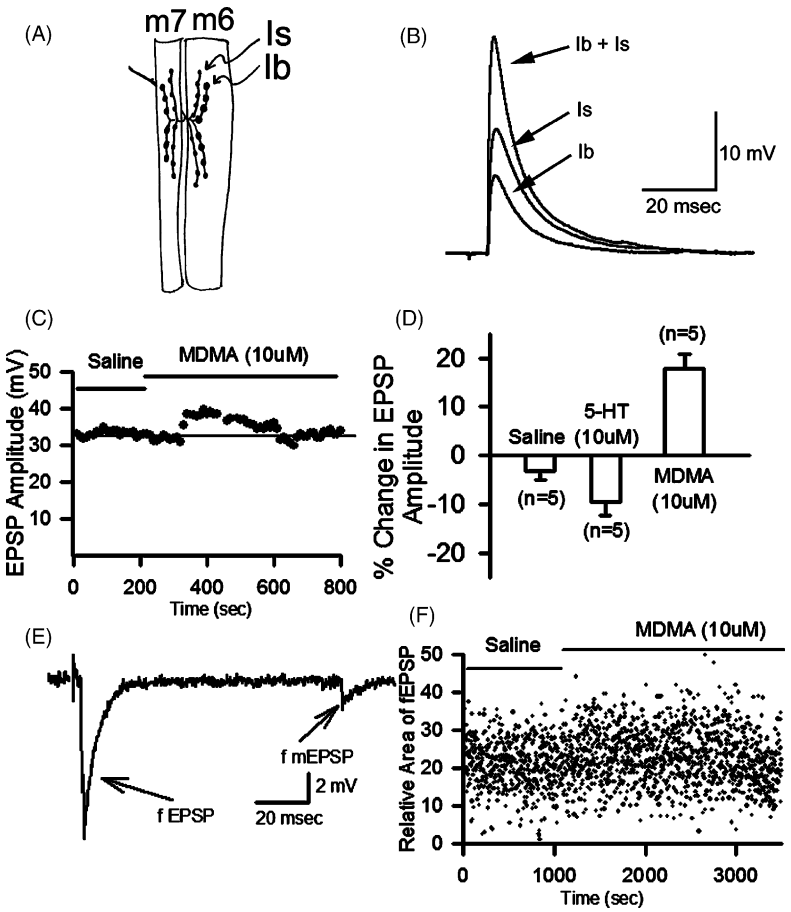

Fig. 4. The *Drosophila* larval neuromuscular preparation. Muscle 6 of the third instar *Drosophila* larvae has two motor nerve terminals that innervate it (A). The Is axon gives rise to large EPSPs in the muscle, where as the Ib axon produces smaller EPSPs (B). MDMA enhances the combined amplitude of the Ib & Is EPSP transiently (C). 10 μM exposure of 5-HT resulted in a slight but non-significant decrease on the amplitude of the composite Ib and Is EPSP when compared to saline controls over the same time period (D). The amplitudes of the EPSPs drop slightly over time in non-voltage clamped *Drosophila* preparations. MDMA (10 μM) produced a slight enhancement of the EPSP amplitude in each preparation examined ($P < 0.05$, $n = 5$ preparations, non-parametric rank sum Wilcoxon test). With focal macropatch recordings over distal Is varicosities the multiquantal evoked release fEPSP and spontaneous quantal events (fmEPSP) are able to be measured (E). An increase in evoked release occurs due to exposure of MDMA (10 μM) (F). Although the changes are small there is a consistent increase in the area of the fEPSPs after exposure to MDMA without any measurable differences in characteristics of spontaneous events.

4. Discussion

Since this study has shown that MDMA enhances synaptic transmission in both the 5-HT sensitive crayfish NMJ and the 5-HT-insensitive *Drosophila* NMJ, the opportunity is ripe for deciphering the physiological sites of mechanisms by which MDMA is functioning at these model synaptic preparations. It appears that MDMA may be working as an agonist at 5-HT receptors in the crayfish and might also be effectively working as an antagonist since if it is applied prior to 5-HT it partially blocks the effects of 5-HT. Dose-response curves for the interaction of the 5-HT and MDMA would help clarify this point and substantiate the claim that MDMA in crayfish activates 5HT receptors. However, with repetitive superfusion of drugs over a given preparation, there will be long exchange times and one would need be concerned about residual effects by second messengers. A full pharmacological quantitative comparison of the drug effects are needed to substantiate the mechanisms in detail. Here, we report on the initial studies in hopes to encourage others to follow suit into the mechanism of MDMA's actions.

The crayfish opener NMJ responds to exogenous application of agonists and antagonists to the vertebrate 5-HT_2 receptor subtypes (Cooper et al., 2001a; Tabor and Cooper, 2002). Injecting a blocking agent to the intracellular phosphatidyl inosotide cascades causes a reduction in the effects of 5-HT in this preparation, reinforcing the analogy between crayfish receptors and vertebrate 5-HT_2 receptor subtypes (Dixon and Atwood, 1989). Agonistic actions on 5-HT receptors in the presynaptic nerve terminal at the crayfish NMJ likely justify the difference between the effects of MDMA on the two species. Since the fly NMJ is insensitive to 5-HT, there may be some other receptor subtype which is not yet known on which MDMA could act; such receptors could also be present at the crayfish NMJ.

In non-human adult mammalian models, MDMA induces toxic effects on central serotonergic neurons at a dose per body weight commonly used by humans. The effect consists of 5-HT depletion and inability to measure 5-HT transporters (Stone et al., 1986; Battaglia et al., 1987; Schmidt, 1987; Ricaurte et al., 1988, 1992). Since the crayfish and *Drosophila* NMJs are not serotonergic, MDMA may not have toxic effects at these glutamatergic NMJs. Our future goals are to investigate long-term effects at these synapses by whole animal studies.

At the crayfish NMJ, exposure to MDMA promoted vesicles to be ready for release within the presynaptic membrane in the absence of electrical activity; the same effect occurs when this NMJ is exposed to 5-HT (100 nM). This is evident in the fact that there are fewer quantal failures with evoked stimulation in the presence of MDMA. Thus, MDMA enhances the probability of a vesicle fusing with the membrane. Since the motor nerve terminals of the crayfish and *Drosophila* NMJs do not store 5-HT, DA, or NE, we can attribute these effects to actions of MDMA and not the potential actions of a transporter for biogenic amines or catecholamines.

Since serotonin increases the number of vesicles that are released with evoked stimulation (Southard et al., 2000; Strawn et al., 2000; Wang and Zucker, 1998), we postulate that MDMA will behave similarly to 5-HT in the crayfish preparation. The signaling molecule Ins(1,4,5)P3 is one such compound that could be key for MDMA's action. IP3 also has other functions within a cell. For example, it can have a direct action on the endoplasmic reticulum (ER) resulting in the release of stored calcium. The calcium from the ER and calcium-induced calcium release processes has a role in synaptic transmission and neuronal function. In fact, ryanodine receptors are now known to be stimulated by cyclic ADP-ribose as well as Ca^{2+}, ryanodine, and caffeine. We recently demonstrated that axonal injections of Adenophostin-A (a stable IP_3 analog) greatly enhances synaptic transmission (Cooper et al., 2001b). These results suggest that the enhanced synaptic response at the NMJ induced by 5-HT might also be caused by activation of intracellular pathways which subsequently release Ca^{2+} from internal stores. There are several direct and indirect signaling pathways within neurons that are known to release calcium ions from organelles that will have an effect on synaptic transmission. In order to elucidate the possibility of internal and/or increased external influx of calcium as potential mechanisms of MDMA's action on the motor nerve terminals, we are now using calcium-sensitive indicators to assay calcium differences among terminals during exposure to MDMA.

Understanding the fundamental mechanisms of the action of MDMA on synaptic performance in these model systems will be directly relevant to all neural systems, including human. In addition, the rapid neural development of the insect model could be used to study developmental issues associated with the effects of MDMA and neural circuits (Cooper, et al. 2003; Dasari and Cooper, 2004). For example, HPLC-determined dopamine concentration decreases from second to third instar larvae (Cooper and Neckameyer, 1999). This decrease likely parallels differences in the density of dopamine receptors. The effects of MDMA through various stages of development may have pronounced effects on other neuromodulatory systems with long-term consequences in adult stages.

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