Physiological separation of vesicle pools in
low- and high-output nerve terminals

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Running Head: Regulation of synaptic vesicles at tonic and phasic NMJs

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Highlights
1. The pools of synaptic vesicles are physiological differentiated in low- and high-output terminals.
 2. The antibiotic bafilomycin A1 blocks re-packaging of transmitter for fast recycling vesicles
3. Electrical activity and/or modulation by 5-HT can recruit the vesicles from a reserve pool.
4. Phasic type NMJs depress faster than tonic nerve terminals due to high percent use of synaptic vesicles.
5. Tonic terminals have a large reserve pool to recruit from with modulation by 5-HT.

Abstract
Physiological differences in low- (tonic like) and high-output (phasic like) synapses match many of the expected anatomical features of these terminals; However investigation in the recruitment of synaptic vesicles from a reserve pool (RP) to a readily releasable pool (RRP) of synaptic vesicles within these types of presynaptic nerve terminals has not been fully addressed. This study highlights physiological differences and differential modulation and/or extent of the vesicles in a RP for maintaining synaptic output during evoked depression of the RRP. With the use of bafilomycin A1, a vacuolar ATPase blocker, recycling vesicles are blocked in refilling with transmitter. The tonic terminal is fatigue resistant due to a large RRP, where as the phasic depresses rapidly upon continuous stimulation. These differences in rates of depression appear to be in the size and degree of utilization of the RRP of vesicles. The working model is that upon depression of the tonic terminal, 5-HT has a large RP to act on in order to recruit vesicles to the RRP; whereas, the phasic terminal, 5-HT can recruit RP vesicles to the RRP prior to synaptic depression but not after depression. The vesicle pools are physiologically differentiated between phasic and tonic output terminals which match behavioral being able to maintain a degree of output level of synaptic efficacy.

1. Introduction

Chemical synaptic communication at neuromuscular junctions (NMJ) generally occurs by synaptic vesicles, packaged with a transmitter, fusing with the presynaptic plasma membrane to release the transmitter into a synaptic cleft for the postsynaptic receptors to receive and respond accordingly. How vesicles fuse with the presynaptic member is an active area of investigation on a comparative scale as it is assumed there are a variety of ways this process may occur from kiss-and-run to full exocytosis of vesicles (Aravanis et al., 2003; Fredj and Burrone, 2009; Rizzoli et al., 2003; Rizzoli and Betz, 2005; Rosenmund and Stevens,1996; Sudhof, 2004). The recycling and repacking of the vesicles are also of substantial interest particularly given that there appear to be different pools of vesicles for various functions within presynaptic nerve terminals and unique processes as well as similarities among various animal species (Atwood and Cooper, 1996a,b; Denker et al., 2011a,b; Rizzoli and Betz, 2005; Sudhof, 2004).

Recently it was suggested that synaptic vesicles may not just serve as a means of packaging transmitter but also providing essential proteins as a buffer source for use when needed (Denker et al., 2011a,b). In addition, a large influx of $Ca2+$ can depress synaptic transmission (Heuser et al., 1971; Katz and Miledi, 1969; Ohta and Kuba, 1980). Also, acidification within the nerve terminals depresses vesicles endocytosis (Lindgren et al., 1997). These processes may serve as potential negative feedback mechanisms. Such observations raise questions about the functional needs of reserve pool (RP) and readily releasable pool (RRP) of vesicles and their role. To determine the functional differences, in terms of vesicle recycling and recruitment in the RP and the RRP between phasic (high-output) and tonic (low-output) motor nerve terminals the packaging of neurotransmitter was pharmacologically blocked in recycling vesicles and the action of the well established modulator serotonin (5-HT) that enhances synaptic efficacy at crustacean NMJ was investigated in this study.

Crustaceans have played a major contribution for investigating structure and function relationships in synaptic transmission that have aided in understanding synapses in general for all animals (Atwood, 1976,1982a,b, Atwood and Cooper 1995,1996a,b; Cooper et al., 1995a,b, 1996a,b; Denker et al., 2011a; Jahromi and Atwood, 1974; Johnstone et al., 2008, 2011; Walrond et al., 1993). An advantage of many NMJs in the crayfish is that they are graded in transmission as many crustacean muscles do not produce action potentials (Atwood, 1967, 1976). This allows one to follow a rise or decrease in synaptic efficacy over time as well as influences in modulation of the synaptic function with quantal analysis (Cooper et al., 1995b,2003; Dudel and Kuffler,1961; Djokaj et al., 2001).

Selective axonal stimulation first studied in crayfish leg extensor yielded two different types of muscle contraction: one a fast twitch-like, the other one with a slower response but depression resistant (Lucas 1907, 1917; Blaschko, et al. 1931; Wiersma, 1933; Van Harreveld & Wiersma, 1936). Later the same contractile pattern and physiology was also identified in crayfish abdomen extensor and flexor musculature (Parnas and Atwood, 1966; Kennedy & Takeda, 1965a,b). Physiological and histological

studies suggested that this difference is not only due to the types of motor neurons (phasic/high-output and tonic/low-output), but also the structure of postsynaptic targets (fast and slow muscles) (Baierlein et al., 2011).

The morphological and physiological differences of tonic and phasic nerve terminals have been studied in the crayfish model (Atwood 1963, 2008) and particularly well in the leg extensor for comparisons (King et al, 1996; Bradacs et al, 1997, Msghina et al, 1998). In this leg preparation both types of nerve terminals innervate the same postsynaptic muscle fiber and give rise to stark differences in postsynaptic responses. An advantage of this preparation is that the target is the same fiber so comparisons in neuron communication can be probed. In this preparation the small varicosities have a high mean quantal content and the synapses depress relatively quickly from the phasic terminals. However, the larger varicosities of the tonic nerve terminal have a low mean quantal content and show marked facilitation with resistance to depression (Wu and Cooper, 2010). Given that the nerve terminals in this preparation do innervate the same fiber there might be feedback from the fiber being stimulated by one neuron to the other non-stimulated neuron or alter receptor sensitivity. So, for our current study we chose to use distinctly separate muscles in the crayfish abdomen that fit phasic and tonic profiles to avoid interaction of the muscle activity.

The abdominal extensor musculature has been well described in *Procambarus clarkii* and other closely related species (Pilgrim and Wiersma, 1963; Parnas and Atwood, 1966; Sohn et al, 2000). All three deep extensor muscles (medial - DEM, lateral 1 - DEL1, lateral 2 - DEL2) are composed of phasic type of muscle fibers (fast-contracting fibers) with short sarcomeres less than 5 µm (Parnas & Atwood, 1966). DEM is a twisted helix muscle. Most of the DEL1 and DEL2 muscle fibers are straight. Kennedy and Takeda (1965a,b) had identified the superficial extensors medial - SEM and lateral - SEL) and their innervation profiles. Both the superficial muscles contain tonic muscle fibers (slow-contracting fibers) with longer sarcomeres ranging 9 µm to 11 µm (Parnas & Atwood, 1966) (see Figure 1 for details).

A recent study of the tonic NMJs on the crayfish opener muscle in the walking leg did demonstrate that blocking the vesicular glutamate transporter (VGlut) with bafilomycin A1 depressed synaptic transmission faster than without its presence and that the rate of synaptic depression is stimulation dependant (Wu and Cooper, 2012a). Also, a working dose of 4µM BA was demonstrated to work well without functional damage for crayfish NMJs. The opener NMJ has a substantial RP which can be recruited by 5-HT application after the induction of synaptic depression, suggesting a functional separation in vesicles of the RRP and RP. In the study, the high-output terminals on the DEL1 muscle fibers were compared to the low-output terminals in SEL muscle fibers within the same segment of abdominal musculature. Based on previous reports of the morphological and physiological characteristics of the tonic and phasic terminals, a slower depression of the tonic terminals with or without BA treatment was predicted. Also, we expected that treatments with BA would depress both the terminals faster than without the drug; however, that phasic terminals would show a much greater rate of depression than tonic terminals in the presence of BA. Given the recent results of tonic terminals on the leg opener NMJs responding to 5-HT after the induction of depression, we expected to be able to recruit vesicles from RP in the tonic terminal to a greater extent than the phasic terminals in the abdominal preparations. Working models

in the functional difference of the vesicle pools in low and high output terminals are presented.

2. Results
2.1 Tonic nerve terminals

When the segmental nerve that innervates SEL muscle fibers was stimulated at 5 Hz in crayfish saline, the average time to 50% depression is 384.4 min (n=5, $\pm$ 13.7 min SEM, Figure 2). Five out of five preparations showed the restoration of synaptic transmission after 5-HT (1 µM) application (p<0.05 non-parametric rank sum test). Preparations responded in varying degrees to the 5-HT application; however, the amplitude of EPSPs were markedly increased in comparison with the depressed state (see Figure 2A example).

2.2 Tonic nerve terminals in 4µM bafilomycin A1

The average time to 50% depression is 58.9 min ($\pm$ 11.8 min SEM, n=5, Figure 2B) using continuous stimulation at 5Hz in the presence of 4µM BA,. As for the preparations bathed in crayfish saline that depressed, all the preparations exposed to 4µM BA showed an enhanced effect to 5-HT by in increasing the EPSP amplitude (p<0.05 non-parametric rank sum test).

2.3 Phasic nerve terminals in crayfish saline

The segmental nerve was stimulated continuously at 5Hz while the very anterior part of DEL1 muscle in the next posterior segment was recorded. By recording in the next segment helps to insure recording the activity from a single phasic motor neuron and not a group of phasic neurons that innervate the same fibers within a segment (See Cooper et al., 1998). The average time to 50% depression time is 7.2 min ($\pm$ 1.2 min SEM, n=5, Figure 2C). After depression, not one of the five preparations showed the rejuvenation of synaptic transmission with exposure to 5-HT, as illustrated in the representative scatter plot (Figure 2C).

2.4 Phasic nerve terminals in 4µM Bafilomycin A1

In the presence of 4µM BA, the phasic nerve terminals depressed much faster. The average time to 50% depression time is 3.8 min ($\pm$ 0.6 min SEM, n=5, Figure 2D). Like in crayfish saline alone for these phasic terminals, exposure to 5-HT did not enhance the synaptic transmission in any of the 5 preparations.

2.5 Overall results

In both tonic and phasic preparations, 4µM BA can depress the terminals markedly faster (Figure 3). After synaptic depression, exposure to 5-HT enhanced synaptic transmission in the tonic NMJs. However, the phasic NMJs which were either exposed or not to BA did not show an increase in EPSP amplitude after 5-HT application.

The phasic NMJs do respond to 5-HT exposure prior to depression, but after depression occurs there is no enhancement (Figure 4). This is a similar finding in 5-HT effects for other phasic NMJs for crayfish (Cooper et al., 2003; Johnstone et al., 2008). In five out of five preparations the phasic NMJs produced an enhanced response to 5-HT application prior to the induction of depression. These five preparations were only used for the 5-HT sensitivity assay prior to depression with minimal stimulation before application of 5-HT with 0.5Hz stimulation for a minute or less in saline and then exposure the saline exchanged to one containing 5-HT. An average percent change in

the EPSP amplitude in response to application of 5-HT for these phasic NMJs is an increase of 82.36% ($\pm$ 19.91 SEM, n=5).

3. Discussion

In this study we have demonstrated that BA, a macrolide antibiotic, produces synaptic depression sooner then without exposure to the drug at the NMJs. Synaptic depression is rapid for the phasic terminals as compared to the tonic terminals; however, after depression of the tonic terminals application of 5-HT rejuvenates the EPSP responses as the amplitude increases. After the high-output phasic terminals fully depress there are apparently no RP vesicles to recruit to the RRP, because no further enhancement by 5-HT was observed. Prior to depression of the phasic terminals, 5-HT does enhance the synaptic responses. Thus, the evidence is suggestive that the high output terminals utilize what might be considered a RP during evoked stimulation. In this case the RP might be considered as a 5-HT sensitive pool as long as synaptic depression has not already occurred. The high-out terminals make use of all available vesicles for maintaining evoked release during the late stages of depression. However, the tonic terminals remain sensitive to enhancing transmission by 5-HT even after substantial depression. A model to explain this phenomenon is that the RP are present in tonic terminal and still available for modulation after depression; however, the RP is small in the phasic and being used during evoked stimulation so there are few, if any, available for modulation after synaptic depression occurs.

Bafilomycin A1 belongs to a family of antibiotics. The "bafilomycins" and "concanamycins" were identified as specific vacuolar ATPase inhibitors (Bowman et al, 1988) and have been proved to be useful pharmacological tools to investigate the function of compartmental acidification, since the hydrogen ion pump is blocked by BA, which prevents repackaging of the synaptic vesicle with transmitter after exocytosis-endocytosis cycle (Cavelier and Attwell, 2007; Kidokoro et al., 2004). The recovery of the tonic nerve terminals by 5-HT after they are treated with BA and subsequent depression implies that mitochondria ATP production is not impaired by BA within the time frame of these experiments because vesicle docking and recycling is an ATP dependent process (Tolar and Pallanck, 1998). Longer exposure times may lead to effects not investigated in this study. We have shown previously that 8 µM BA appears to have some unwanted effects in a crayfish NMJ as the nerve would fail to conduct electrical signals; although, motor neurons function in larval *Drosophila* NMJs even up to 16 µM (Wu and Cooper, 2012a). Generally, BA appears to work as suggested for crayfish NMJs (Wu and Cooper 2012a,b) since the results are fitting in a dose dependent manner and for incubation times as well as synaptic responses as for *Drosophila* NMJs (Denker et al., 2011a; Kuromi and Kidokoro, 2000) and mammalian brain slices (Cavelier and Attwell, 2007). So this compound can likely be utilized in other crustacean models for similar experimental manipulations.

The action of 5-HT at NMJs in a variety of invertebrates reveals a range of responses from excitation to inhibition depending on the species (Wu and Cooper, 2012b). However, at crayfish NMJs 5-HT has always been demonstrated to enhance synaptic transmission (Florey and Florey, 1954; Fischer and Florey, 1983; Dudel 1965), for the inhibitory (GABA) as well as excitatory (glutamate) motor neurons (Johnstone et al., 2008; Vyshedskiy et al., 1998; Wang and Zucker, 1998). The tonic like walking leg

opener muscle in crayfish responds to 5-HT with an increase in the EPSP amplitudes of the excitatory nerve. This effect is mostly due to an increase in the mean quantal content related to more synaptic vesicles docking and fusing (Cooper et al., 2001; Djokaj et al., 2001; Logsdon et al., 2006; Sparks and Cooper, 2004). Likewise, in the walking leg of crayfish, the extensor muscle is innervated by phasic and tonic terminals and both motor nerve terminals increase in synaptic efficacy with 5-HT application (Cooper et al., 2003). However the tonic terminal shows a higher sensitivity to 5-HT as compared to the phasic even when the basal synaptic output is decreased by lowered extracellular Ca2+ (Cooper et al., 2003). Even though the phasic terminals are not maximized in vesicle fusion with lower evoked release, due to lowered bathing calcium, the responses do not appear to maximize the synaptic output with 100 nM 5-HT. In addition, the percent increase in synaptic output was similar as the normal bathing saline (13.5 mM) as for the saline containing the reduced Ca2+(6.75 mM), suggesting that a given activation of receptors or involvement of second messenger systems paralleled the two conditions.

Recently it was demonstrated that the 5-HT mediated responses at crayfish NMJs is in part mediated by PLC. Blocking PLC activation by pretreatment of U73122 (50µM) solution drastically reduced the enhancement of the EPSP amplitude observed with 5-HT application (Wu and Cooper, 2012b). Investigation of PLC inhibition and 5-HT treatment for phasic terminals has not been approached yet; however, we expect a dampened response with such inhibition. The phasic terminals in the leg extensor do show an enhancement in the EPSP amplitude prior to synaptic depression by application of 5-HT (Johnstone et al., 2008), just as for the abdominal extensor muscle used in the herein study. Likewise, little enhancement occurs by 5-HT application after synaptic depression is induced in either of these phasic motor terminal preparations. A descriptive model helps to visualize what appears to be the differences in recruitment of vesicles from a dynamic RP, or for argument sake, vesicles that are sensitive to being recruited by modulation of downstream effects induced by 5-HT for tonic and phasic like motor nerve terminals. Figure 5 and 6 highlight a physiological model of RP and RRP vesicle utilization with evoked stimulation based on the observations to date in tonic low-output and phasic high-output NMJs of the crayfish model. Due to the large enhancement in the EPSP amplitude induced by 5-HT prior or after synaptic depression occurs, the prediction is that there is a larger RP of vesicles for the tonic terminals. This is also supported by the fact that BA inhibits the recycling pool from repackaging with neurotransmitter and a RP can still be recruited by 5-HT. However, for the phasic terminals if synaptic depression is induced by evoked stimulation with or without BA treatments there is little if any RP to be modulated by downstream actions of exposure to 5-HT. Considering, that high-output terminals in multiple types of crayfish preparations are slightly enhanced, as compared to tonic terminals, in synaptic efficacy by 5-HT prior to evoked synaptic depression may indicate that a small RP of vesicles exists in phasic terminals but are rapidly utilized during evoked activity of the terminal. Given that treatment with BA produces rapid depression in high-output terminals is indicative that a large percentage of vesicles are utilized with each stimulation in the terminal.

As to why such differences in sensitivity to 5-HT occurs for evoking an enhancement of the EPSP amplitudes between the tonic and phasic terminals further

investigation into 5-HT receptor density, subtypes, and second messenger recruitment as well as potential intracellular Ca2+ release from the endoplasmic reticulum needs to be addressed. Different 5-HT receptor subtypes use varying cellular cascades (Hensler, 2002) and it is known that there are differences in the amount of the calcium binding protein frequenin (Jeromin et al., 1999) in tonic and phasic terminals of crayfish as well as differences in and the amount of Ca2+ influx with evoked stimulation (Msghina et al., 1999). The varying degree of Ca2+ entry with stimulation may have a role in the recruitment of RP into the RRP for the phasic terminals since the Ca2+ is greater. For potential roles of second messenger systems induced by 5-HT or Ca2+ in crayfish motor nerve terminals see earlier reports (He et al., 1999; Tabor and Cooper, 2002; Wu and Cooper, 2012a,b). As for potential mechanisms in evoked stimulation induced recruitment of vesicles, in the absence of exogenous neuromodulation, see reports by Akbergenova and Bykhovskaia,(2009), Aravanis et al., (2003), Denker et al., (2012a,b), Desai-Shah and Cooper, (2009, 2010), Fiumara et al., (2004), Kuromi and Kidokoro (2000), Sudhof (2004) and Yamashita (2012). The usage of the RRP and RP of these tonic and phasic NMJs in the crayfish abdomen are fitting to their physiological profiles for the musculature involved in rapid tail flips or slow movements in postural control (Cooper et al., 1998; Mykles et al., 2002).

4. Experimental Procedure

4.1 General. All the experiments were carried out in the midsize crayfish (*Procambarus clarkii*) measuring 6-10 cm in body length. They were individually housed in plastic containers with oxygenized water. The temperature of the animal room was controlled at 20°C-21°C. The animals were fed with dry fish food and water changed on weekly basis.

4.2 Dissection. The thorax was removed by a cross-section between thorax and abdomen. To expose the abdomen extensor muscles, the ventral side was removed after cutting along the lateral midline on both sides of the abdomen, to reveal the ventral surface of the extensor musculature. The dorsal part was completely spread out and pinned down in a sylgard dish to reduce twitching during stimulation. All the connective tissues and residual flexor muscles were removed to better visualize DEL1, DEL2, DEM and SEL. Only DEL1 and SEL in segment A2, A3, A4 were used through the whole experiments (Figure 1). Dissected preparations were maintained in crayfish saline, a modified Van Harreveld's solution (in mM: 205 NaCl; 5.3 KCl; 13.5 $CaCl_2 2H_2O$; 2.45 $MgCl_2 6H_2O$; 5 HEPES adjusted to pH7.4).

4.3 Pharmacology. All chemicals were obtained from Sigma-Aldrich Chemical (St. Louis, MO). BA(B1793) solution were made by dissolving 10µg powder in 20µl DMSO (99.9%), then adding crayfish saline to the desired concentrations based on experimental conditions. The solution was stored at -20°C and used within 3 months. 4µM BA, 2.5hrs incubation was used based on the dose-response relation found in leg opener preparation of crayfish (Wu and Cooper, 2012a). The preparations were incubated in BA solution for 2.5hrs, then stimulated using a suction electrode. During the 2.5hrs incubation time, the BA solution was circulated in the dish every 30mins. 1µM

5-HT was made in crayfish saline from frozen stock of 1 mM 5-HT. It was added to the preparation to exchange with BA solution after synaptic depression occurred.

4.4 Physiology. Only segments A2, A3 and A4 were used. The nerve bundles were stimulated in one segment above for phasic muscle (DEL1) recording or in the same segment for tonic muscle (SEL) recording by a suction electrode connected to a Grass stimulator. The effects of BA on the rate of synaptic depression were compared between the phasic and tonic nerve terminals using continuous stimulation at 5Hz. In the 5-HT sensitivity assay prior to depression, the nerve was stimulated at 0.5Hz while DEL1 in the same segment was recorded immediately for 1min or less in saline then after the application of 5-HT. EPSPs were recorded following standard procedures (Baierlein et al., 2011; Cooper et al, 1995a; Crider and Cooper, 2000). Electrical signals were recorded online to a computer with Chart or Scope version 5 software (ADInstruments) via a PowerLab/4s (ADInstruments) interface, respectively.

4.5 Analysis. The amplitude of the last EPSP every 5mins or 1min in tonic preparations and ever 1 sec in phasic preparations was measured. Scatter plots of the EPSP amplitudes were graphed. The time to reach a 50% reduction in the peak EPSP amplitude was used as an index to calculate the half depression time. The averages of times to 50% decline were compared between phasic and tonic nerve terminals. For the 5-HT effect before synaptic depression, the percentage EPSP amplitude change before and after adding 5-HT was calculated.

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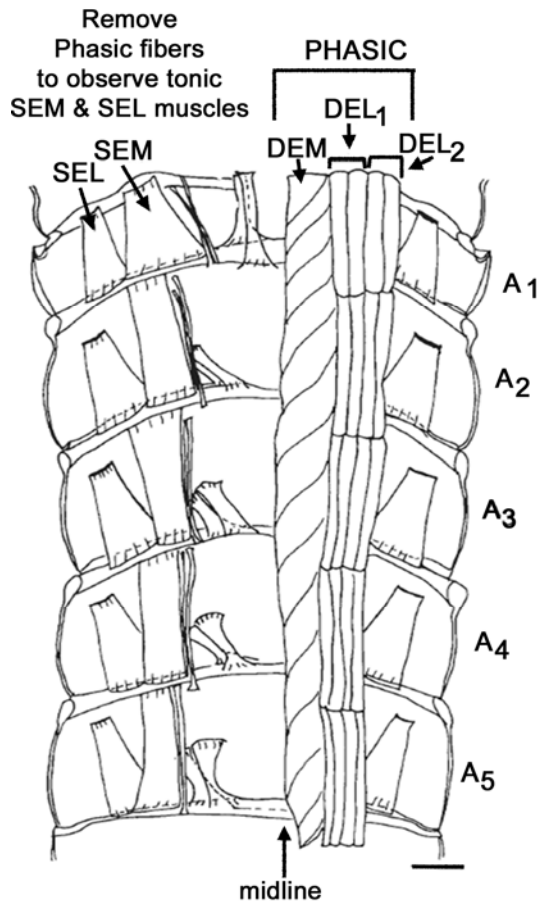
Figure 1: Schematic presentation of crayfish abdomen extensor musculature. Each side of each segment contains deep extensor medial muscle (DEM), deep extensor lateral muscle 1 (DEL1), deep extensor lateral muscle 2 (DEL2), superficial extensor lateral muscle (SEL), superficial extensor medial muscle (SEM). On the left side of the figure, dorsal SEL, SEM is viewed by removing DEM, DEL1, and DEL2. DEM, DEL1 and DEL2 are phasic muscles whereas SEM and SEL are tonic in nature. A1-A5 means abdomen segments. Scale bar = 2.35 mm. The figure is modified from Sohn et al, 2000.
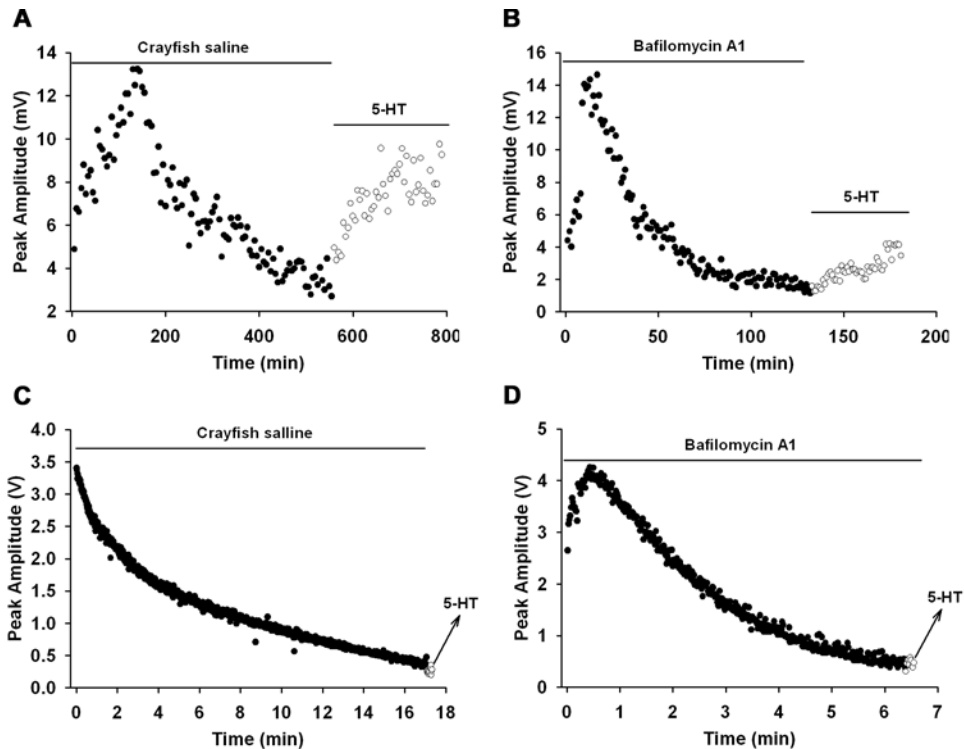
Figure 2: The synaptic transmission of abdomen tonic and phasic nerve terminals in crayfish saline and a 4µM BA containing saline. (A) Representative scatter plot of EPSP amplitude (mV) over time for saline control in tonic terminals. After substantial depression was observed, 5-HT containing saline was exchanged for the saline bath. This resulted increasing amplitudes of the EPSPs. (B) The synaptic activity of abdomen tonic nerve terminals in 4µM BA. The EPSPs depress sooner than controls and after substantial depression exposure to 5-HT also produced an increase in the EPSP amplitude. (C) The synaptic activity of abdomen phasic nerve terminals in crayfish saline depress rapidly as indicated by the decline in EPSP amplitude. After substantial depression was observed exposure to 5-HT did not produce an increase in the EPSP amplitude. (D) The EPSP amplitudes produced by the abdomen phasic nerve terminals decreased faster than saline controls when incubated with 4µM BA. As with the saline control preparation, the EPSPs did not restore with subsequent exposure to 5-HT after depression occurred.
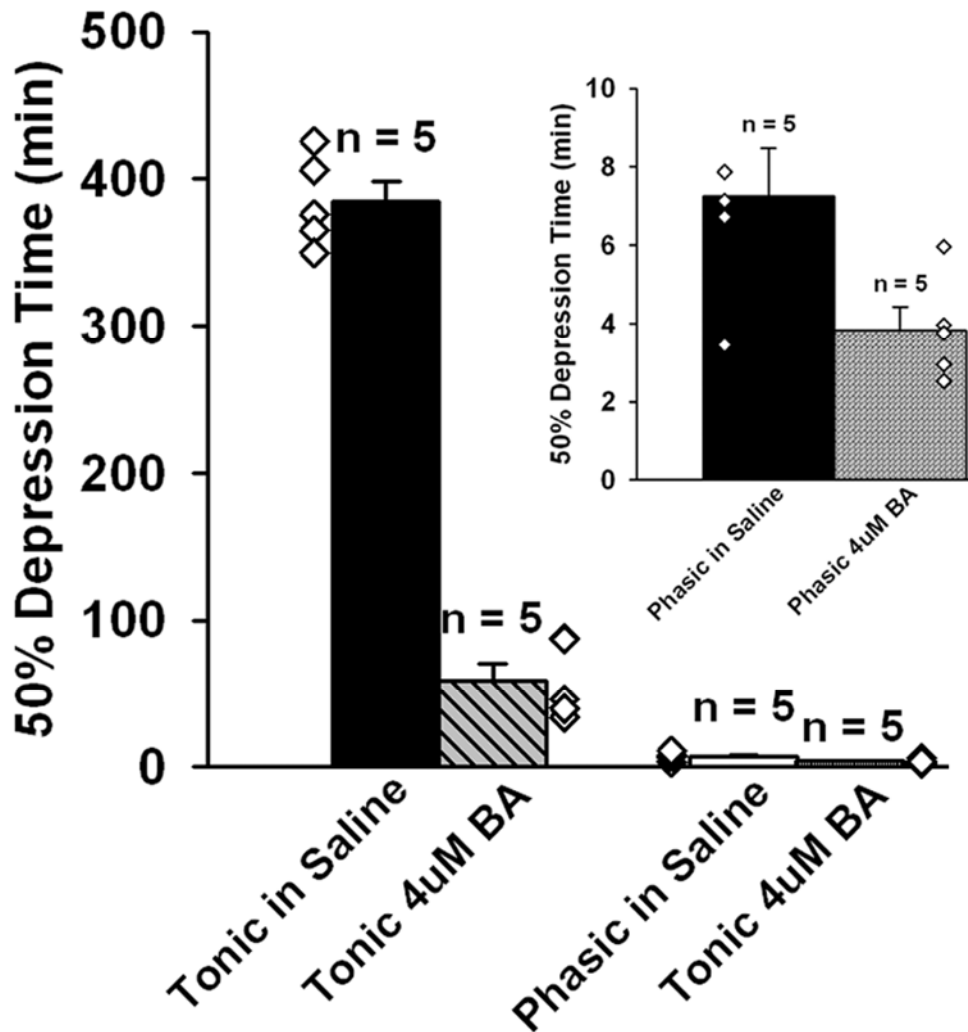
Figure 3: Bar graphs depicting the average time to 50% depression time in the EPSP amplitude for 5 preparations. The average depression time is 384.4 min (± 13.7 min SEM, n=5) for tonic NMJs exposed to saline only. When the tonic NMJs are incubated in BA the depression time is 58.9 min (± 11.8 min SEM, n=5). The phasic terminals treated as a saline control depressed on average 7.2 min (± 1.2 min SEM, n=5,). When the phasic terminals were treated with BA they depressed on average 3.8 min (± 0.6 min SEM, n=5). The diamonds are the values for each individual preparation. The enlarged inset in the bar chart for the phasic terminals expanded as compared to the plot with the same time scale for the phasic and tonic NMJs.
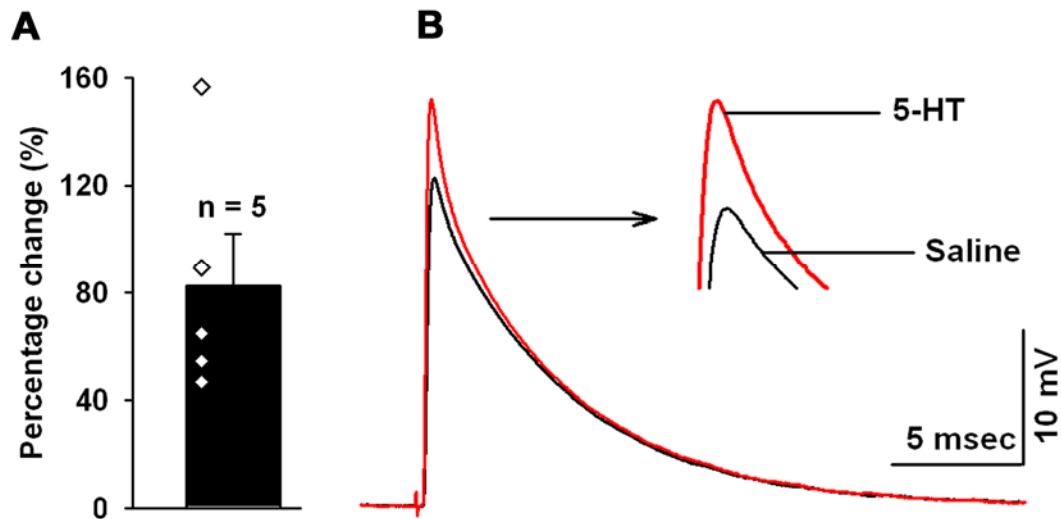
Figure 4: The phasic high-output NMJs response to 5-HT prior to evoked depression. Five out of five preparations show an enhancement in the EPSP amplitude to application of 5-HT (A). The increase in peak amplitude of the EPSP is obvious prior to synaptic depression (B). This is not the case after the phasic terminals are depressed by prolonged activity.
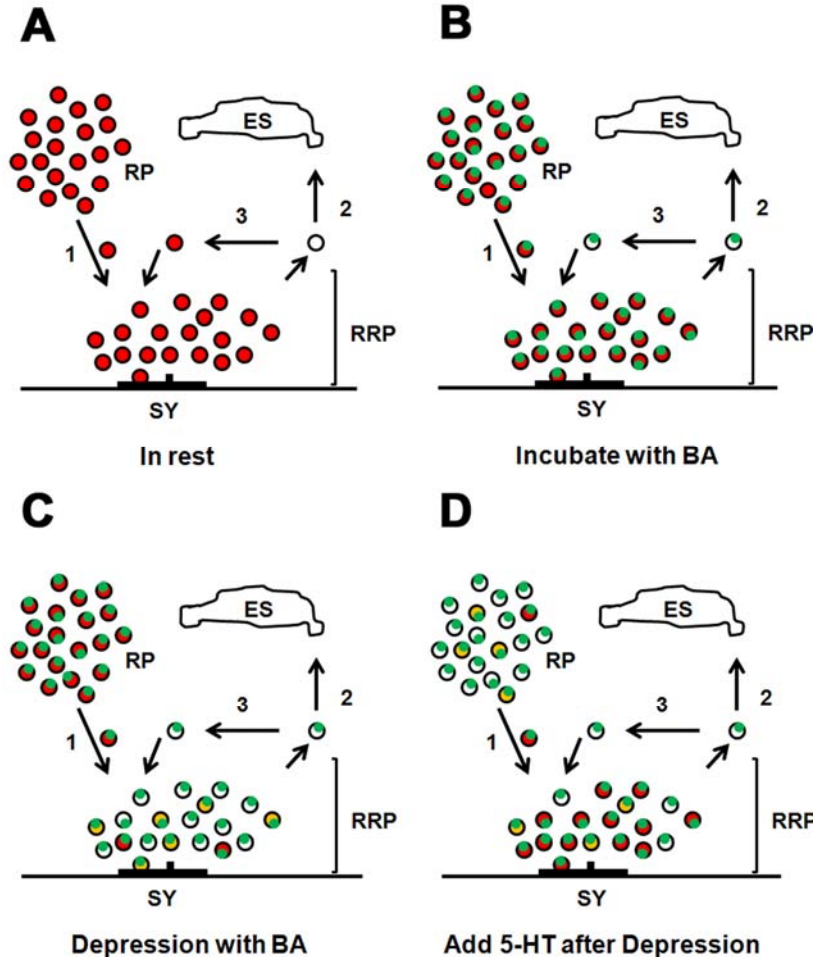
Tonic Low-output Terminal

Figure 5: Model of vesicle recycling between RP and RRP and the effect of BA and 5-HT on transmitter release with the associated vesicle dynamics in low-output NMJs. (A) Synaptic vesicles are separated into RRP and RP over a synapse and with electrical stimulation vesicles in RP can slowly join in to the RRP (1), and then recycle back to RP either through or bypass endosome (2 or 3). (B) In an active synapse, in addition to the slow recycling loop, vesicles in RRP recycle quickly within the RRP which is named quick recycling loop (3). Recycling vesicles are refilled with glutamate (red vesicles are filled with glutamate) but incubating with BA (green dots represent BA) the recycling vesicles can not refill (clear vesicles). (C) With repetitive stimulation the vesicles in the RRP are depleted of glutamate and with BA the packaging is depressed at considerable faster. (D) However application of 5-HT recruits vesicles from the RP to the RRP. In the presences of BA the RRP and vesicles recycling to the RP will become depleted of transmitter. These low output synapses have a larger RP to recruit from than the high-output synapses. RP, reserve pool; RRP, readily releasable pool; SY, synapse; BA, bafilomycin A1; ES, endosome.
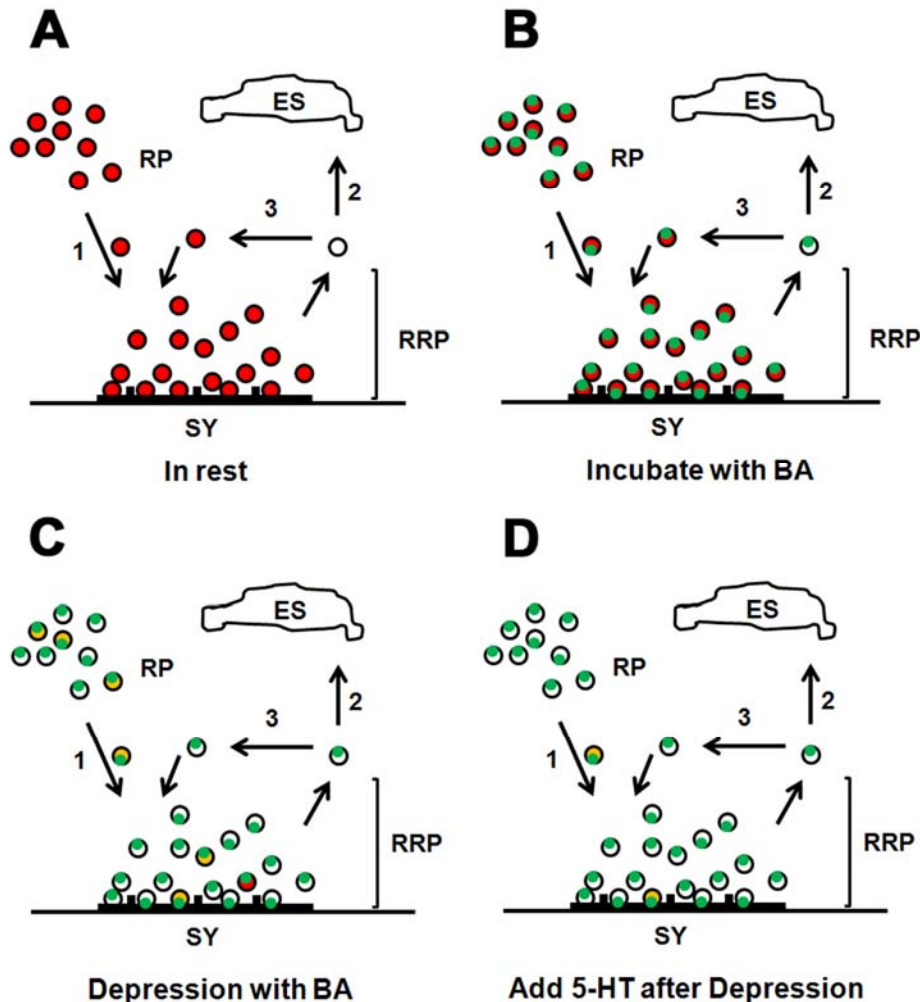
Phasic High-output Terminal

Figure 6: Model of vesicle recycling between RP and RRP and the effect of BA and 5-HT on transmitter release with the associated vesicle dynamics in high-output NMJs. (A) Synaptic vesicles are separated into RRP and RP over a synapse and with electrical stimulation vesicles in RP can slowly join in to the RRP (1), and then recycle back to RP either through or bypass endosome (2 or 3). (B) In an active synapse, in addition to the slow recycling loop, vesicles in RRP recycle quickly within the RRP which is named quick recycling loop (3). Recycling vesicles are refilled with glutamate (red vesicles are filled with glutamate) but incubating with BA (green dots represent BA) the recycling vesicles can not refill (clear vesicles). Since the high-output synapses have more vesicles in the RRP a larger postsynaptic effect is observed. (C) With repetitive stimulation the vesicles in the RRP are depleted of glutamate and with BA the packaging is depressed at considerable faster rate. Since more vesicles are recycling the pool is affected at a greater rate that for low-output synapses. (D) However application of 5-HT recruits vesicles from the RP to the RRP (A or B plates). However, after synaptic depression occurs the vesicles in RP as well as the RRP are depleted of glutamate. Hence, 5-HT has little effect in recruiting any packaged vesicles from the RP.

RP, reserve pool; RRP, readily releasable pool; SY, synapse; BA, bafilomycin A1; ES, endosome.