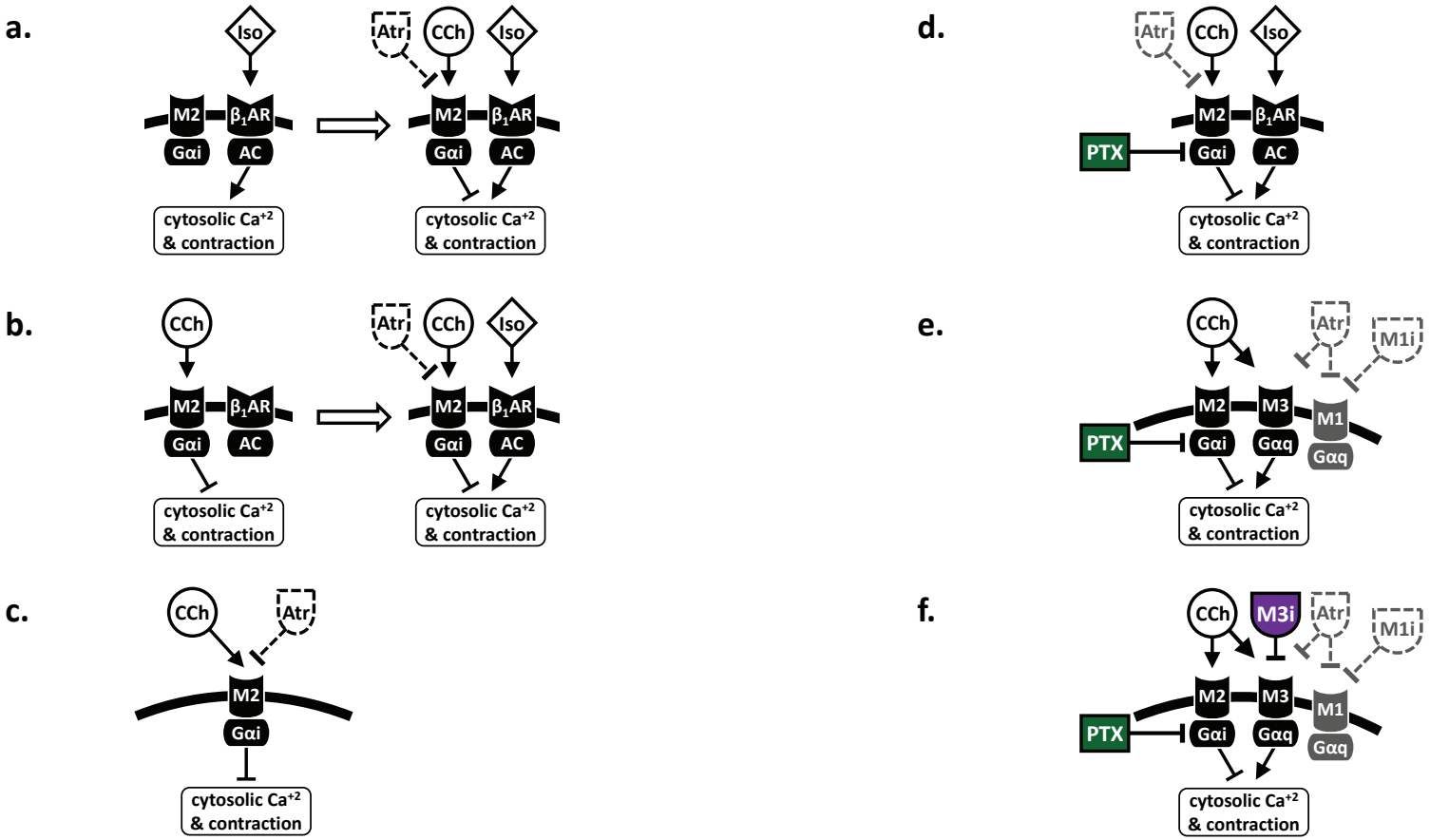
Supplemental Materials

DeMazumder et al., Cardiac resynchronization therapy restores sympathovagal balance in the failing heart by differential remodeling of cholinergic signaling. Circulation Research (2015)

ONLINE FIGURE I: Schematic index of protocols employed for functional experiments on myocytes isolated from the LV lateral wall



Each panel shows the schematic of a cardiomyocyte cell membrane containing β-adrenergic (β_1-AR) and/or muscarinic (M1, M2, M3 mAChR) receptors coupled to intracellular second messengers [Gαi, Gαq, or adenylate cyclase (AC)]; the specific stimulators and/or inhibitors corresponding to the receptors that were employed in the solution exchange protocol are on top; the measured steady-state responses (calcium transient, sarcomere shortening) are at the bottom.

a. Each myocyte was exposed first to isoproterenol (Iso) to stimulate β_1-AR. Then, carbamylcholine (CCh) was added to stimulate mAChRs in the continued presence of Iso. Finally, atropine (Atr) was added to assess reversal of mAChR-specific effects. The data from this protocol are shown in **Fig. 2a-c** and **Online Fig. 2a-b**.

b. Similar to panel a, each myocyte was exposed first to CCh, then Iso in the continued presence of CCh, and finally Atr (data shown in **Fig. 3a-c** and **Online Fig. 3a-b**).

c. The myocyte was first exposed to CCh alone followed by Atr (**Fig. 4a-c** and **Online Fig. 4a-b**).

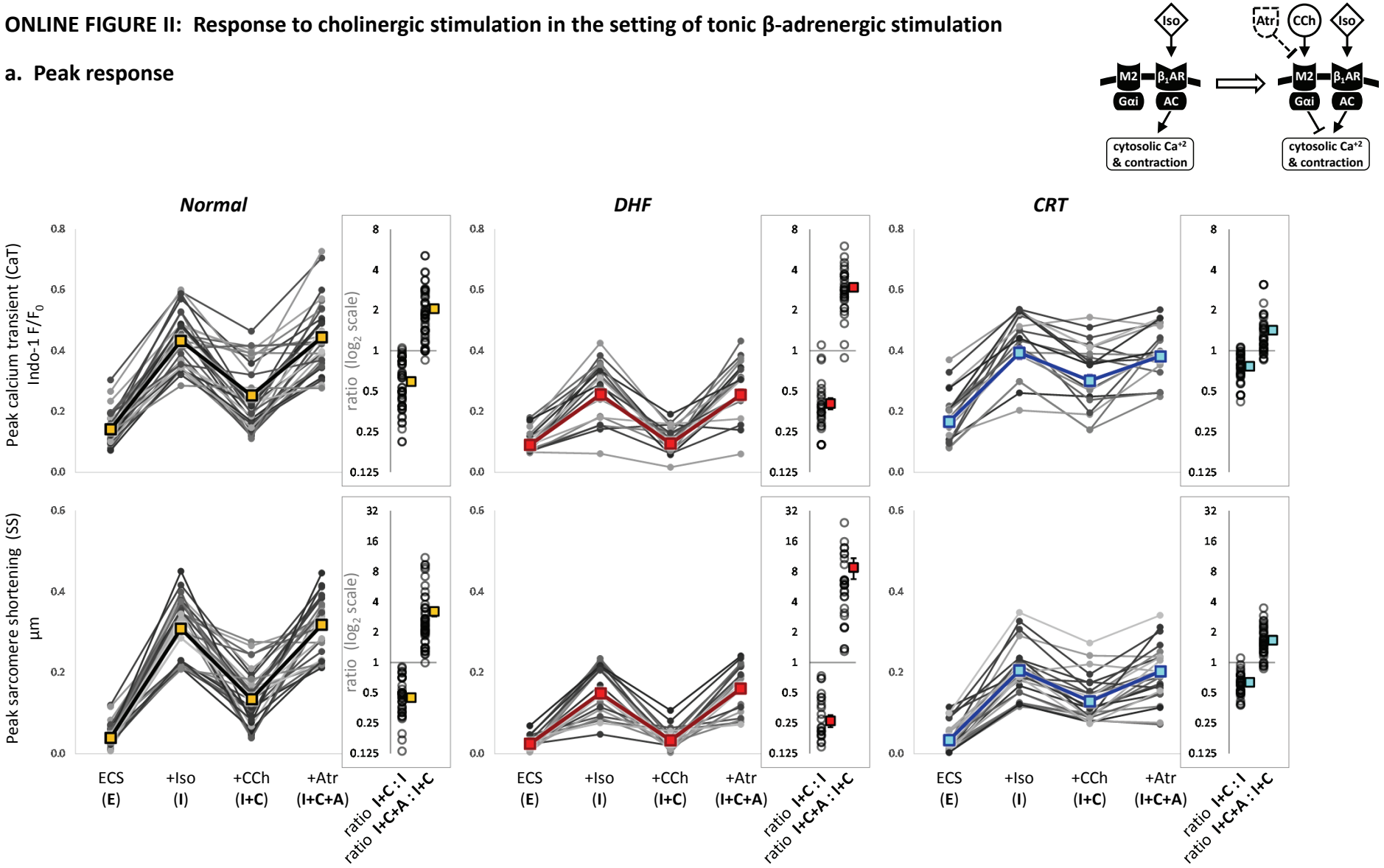
d. The protocol was same as in panel a, except that the experiments were performed in the presence of pertussis toxin (PTX; Gαi inhibitor) (**Fig. 5c**, **Fig. 6a** and **Online Fig. 5a**).

e. The protocol was same as in panel c, except for the presence of PTX and M1-mAChR-specific inhibitor (M1i) to assess for mAChR-stimulated effects not mediated by M2-Gαi and M1-Gαq (**Fig. 6b** and **Online Fig. 5b**).

f. The protocol was same as in panel e, except for the presence of M3-mAChR-specific inhibitor (M3i). Comparison of these data with those from panel e were used to assess for effects specific to the M3-mAChR (**Fig. 6b** and **Online Fig. 5c**).

ONLINE FIGURE II: Response to cholinergic stimulation in the setting of tonic β-adrenergic stimulation

a. Peak response

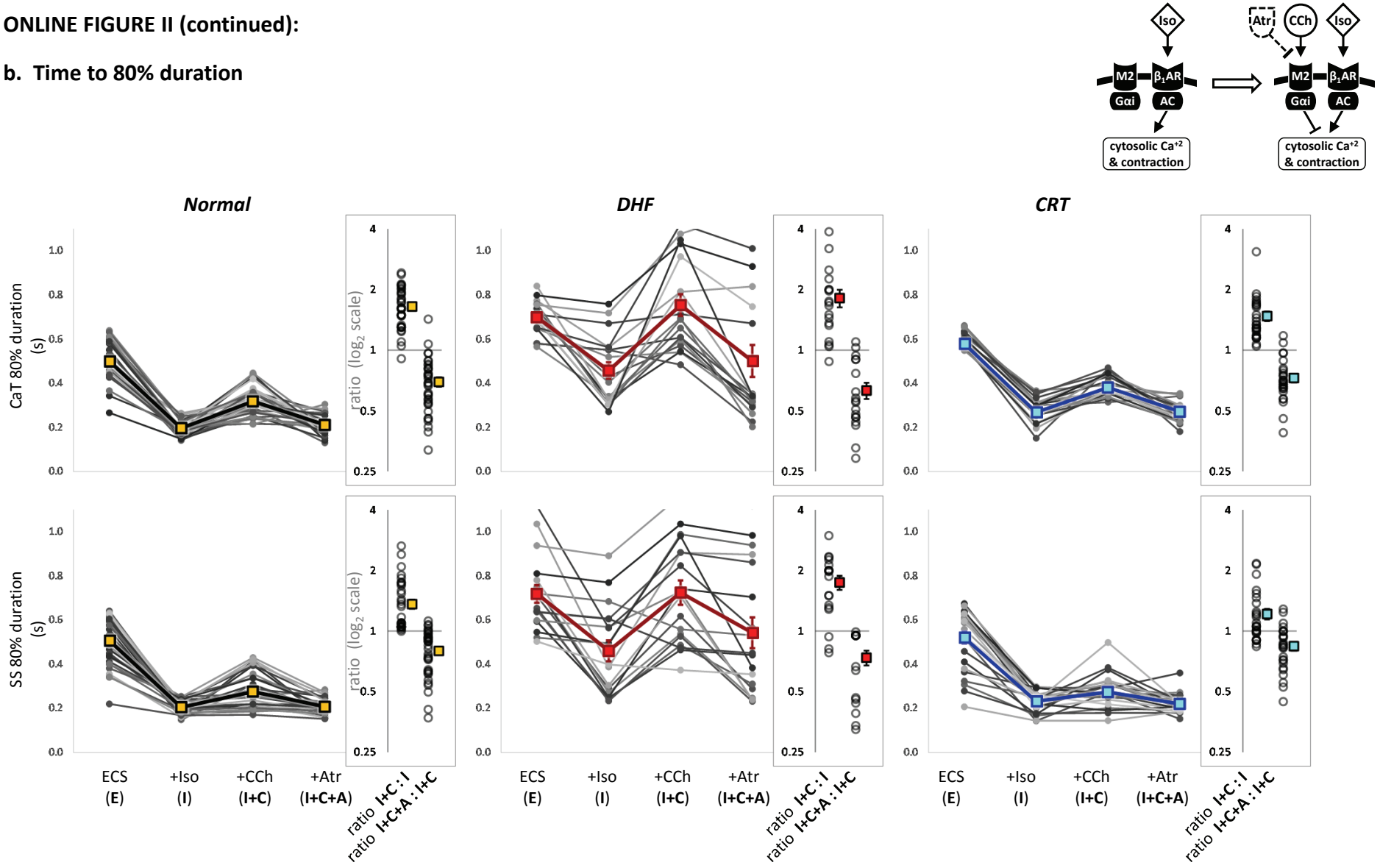


For each individual LV cardiomyocyte (grey filled circles) from normal controls (left column), DHF (middle) and CRT (right) animals (N=6-9 hearts/group), the peak CaT (top row) and SS (bottom) amplitudes are plotted for sequential exposures to ECS (E), Iso alone (I), Iso+CCh (I+C), and Iso+CCh+atropine (I+C+A). For each LV cardiomyocyte within groups, the inset (log_2 scale; grey empty circles) plots the ratio of the responses to CCh added to Iso compared to Iso alone (I+C:I) as well as the ratio of I+C+A:I+C. The corresponding mean±SEM values are indicated by the colored filled markers.

The I+C+A:I+C ratio was larger than the I+C:I ratio within each inset for all groups ($p<0.001$); these ratios were also distinct between groups ($p<0.001$).

ONLINE FIGURE II (continued):

b. Time to 80% duration

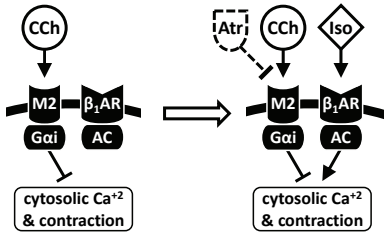


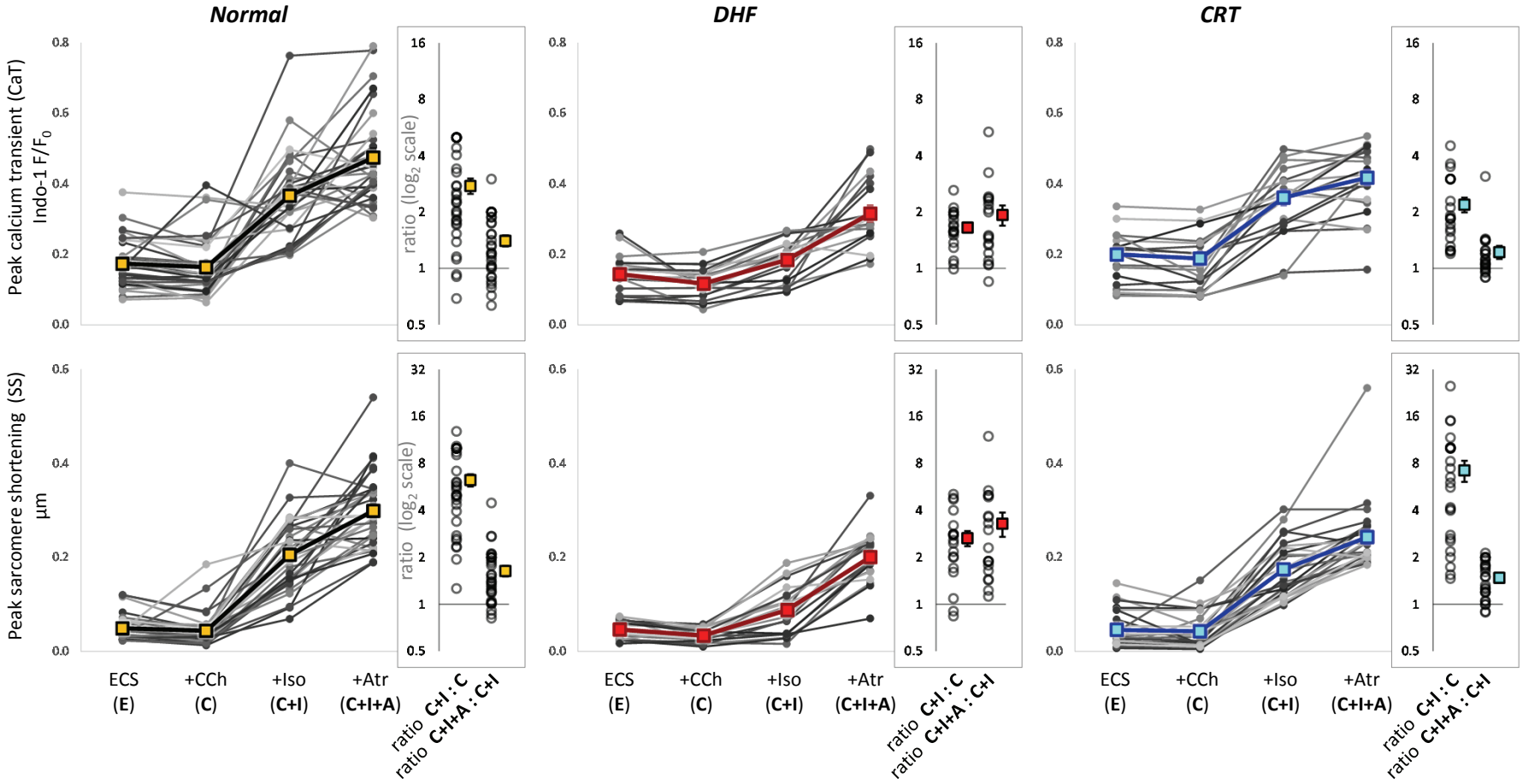
The 80% durations of the CaT (top row) and SS (bottom) are plotted in a format similar to that in Online Figure 2a.

The I+C+A:I+C ratio was larger than the I+C:I ratio within each inset for all groups ($p<0.01$). The SS I+C:I ratio in DHF was larger than those in normal or CRT ($p<0.01$).

ONLINE FIGURE III: Response to β-adrenergic stimulation in the presence of tonic cholinergic stimulation

a. Peak response



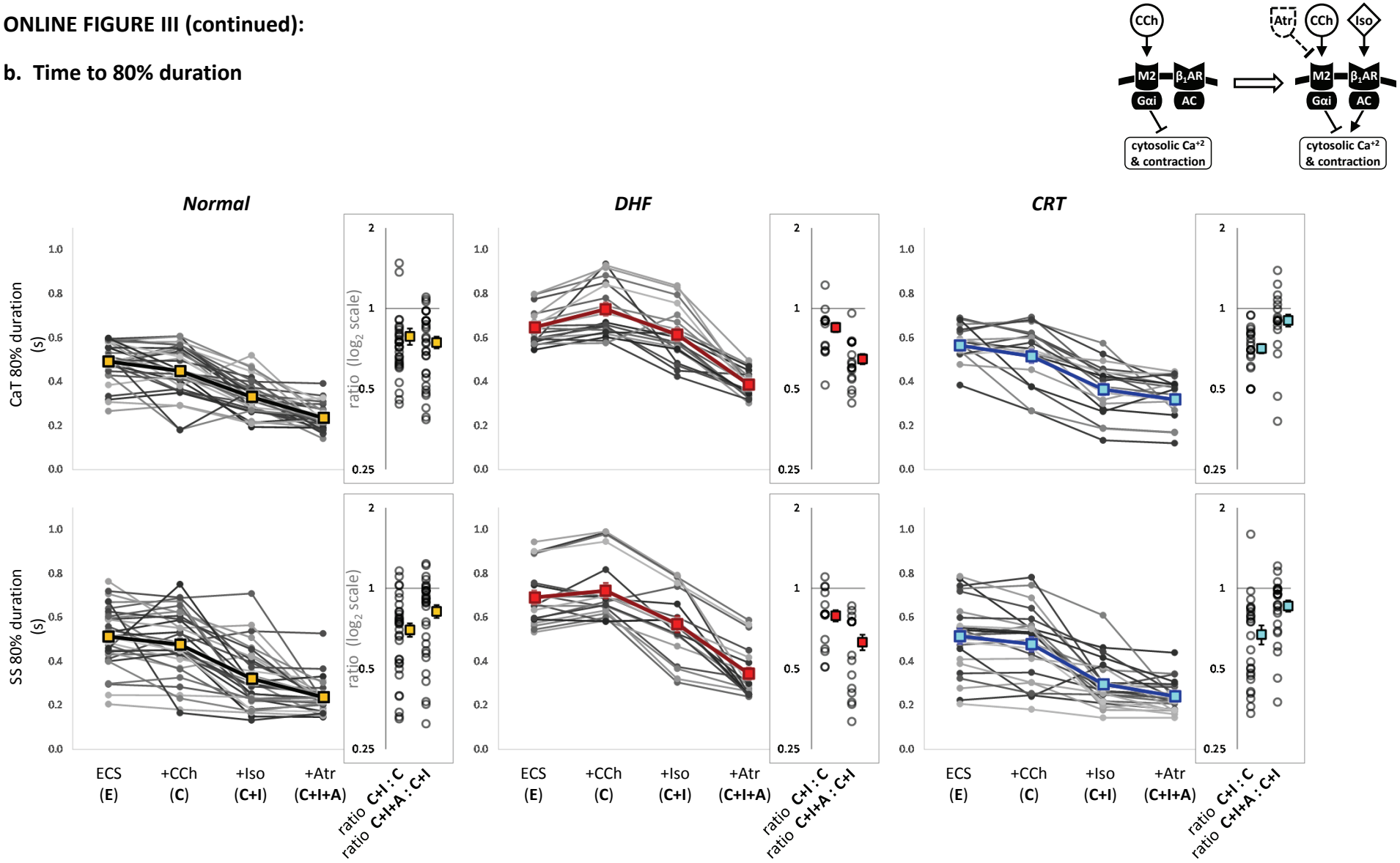


For each individual LV cardiomyocyte (grey filled circles) from normal controls (left column), DHF (middle) and CRT (right) animals (N=6-9 hearts/group), the peak CaT (top row) and SS (bottom) amplitudes are plotted for sequential exposures to ECS (E), CCh alone (C), CCh+Iso (C+I), and CCh+Iso+atropine (C+I+A). For each LV cardiomyocyte within groups, the inset (log_2 scale; grey empty circles) plots the ratio of the responses to Iso added to CCh compared to CCh alone (C+I:C) as well as the ratio of C+I+A:C+I. The corresponding mean±SEM values are indicated by the colored filled markers.

The I+C+A:I+C ratio was larger than the I+C:I ratio within each inset in normal and CRT ($p<0.001$) but not in DHF. Whereas the C+I:C ratios for the CaT and SS in DHF were smaller than those in normal or CRT ($p<0.01$), the C+I+A:C+I ratios in DHF were larger ($p<0.05$).

ONLINE FIGURE III (continued):

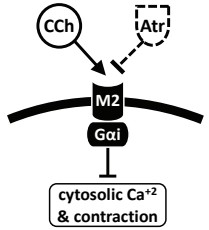
b. Time to 80% duration



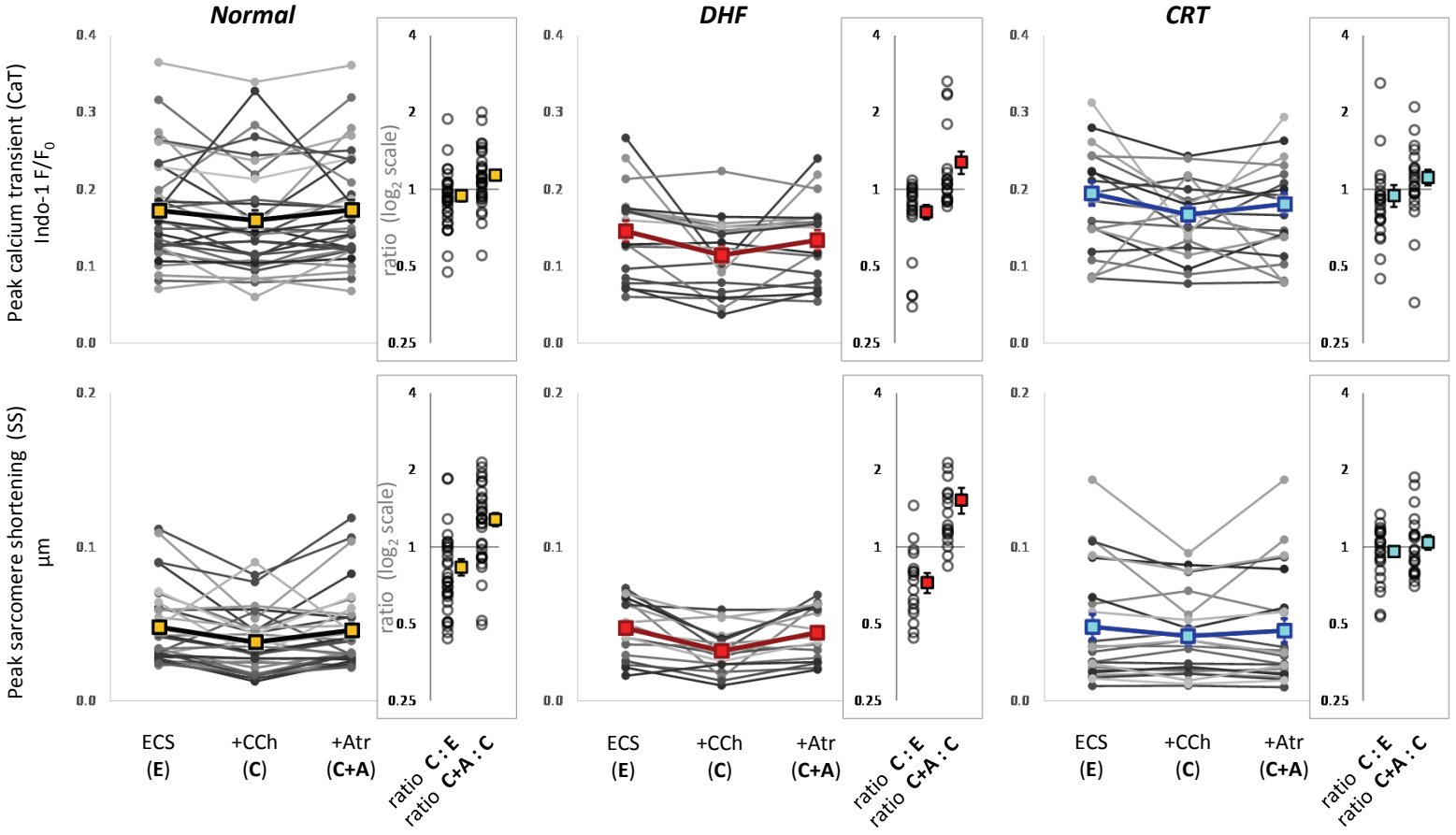
The 80% durations of the CaT (top row) and SS (bottom) are plotted in a format similar to that in Online Figure 3a.

The C+I+A:C+I ratio and the C+I:C ratio were significantly different within each inset in DHF and CRT ($p<0.05$), but these ratios were similar in normal controls. Whereas the C+I+A:C+I ratio was smaller than the C+I:C ratio in DHF, the converse was seen in CRT ($p<0.01$).

ONLINE FIGURE IV: Cholinergic stimulation alone in normal, DHF and CRT



a. Peak response

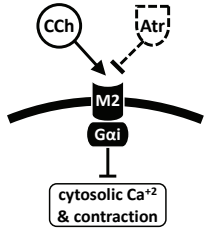


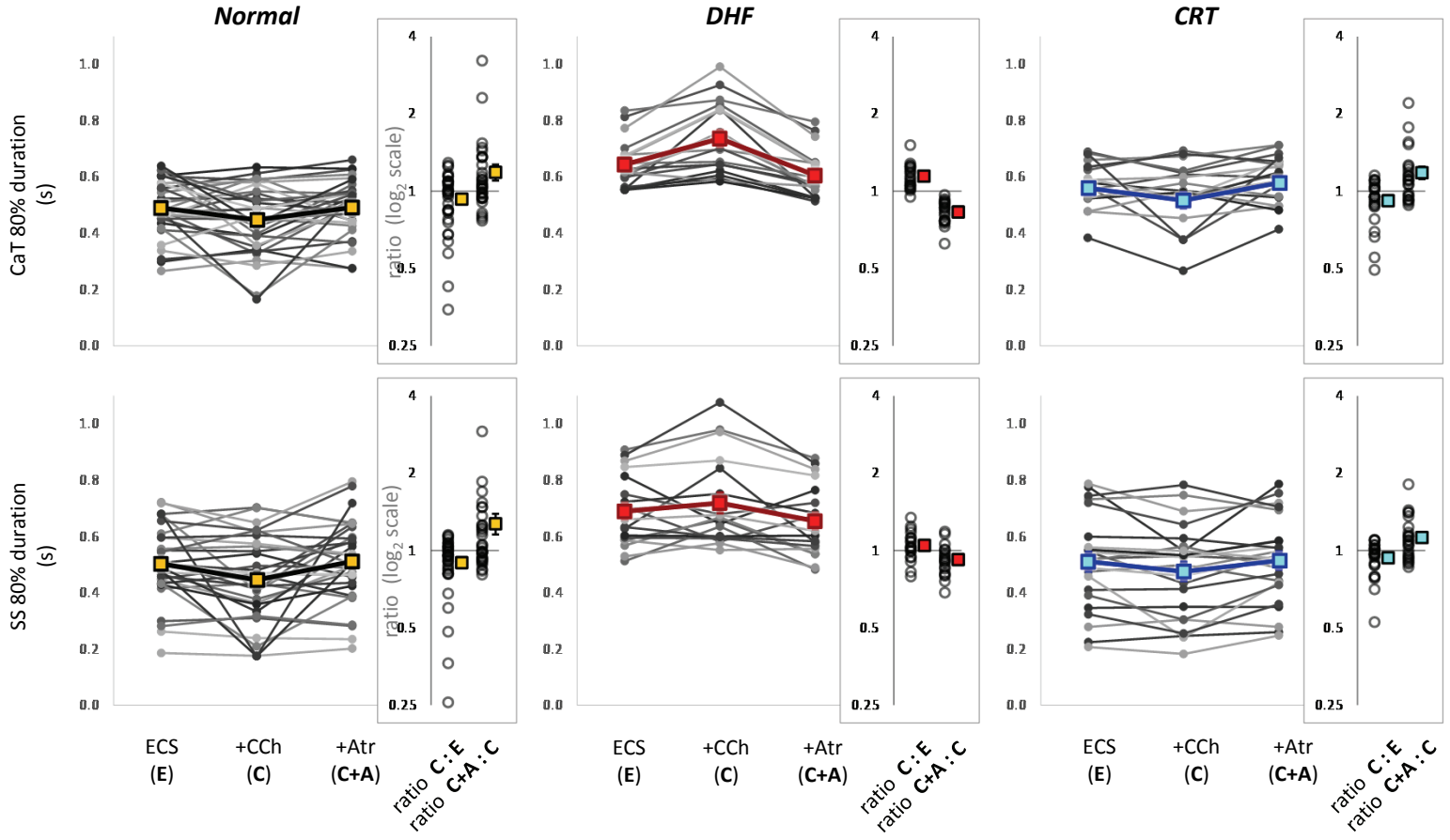
For each individual LV cardiomyocyte (grey filled circles) from normal controls (left column), DHF (middle) and CRT (right) animals (N=6-9 hearts/group), the peak CaT (top row) and SS (bottom) amplitudes are plotted for sequential exposures to ECS (E), CCh alone (C), and CCh+atropine (C+A). For each LV cardiomyocyte within groups, the inset (log_2 scale; grey empty circles) plots the ratio of the responses to CCh compared to ECS alone (C:E) as well as the ratio of C+A:C. The corresponding mean±SEM values are indicated by the colored filled markers.

The C+A:C ratio was larger than the C:E ratio within each inset in normal and DHF ($p<0.05$) but these ratios were similar in CRT.

ONLINE FIGURE IV (continued):

b. Time to 80% duration



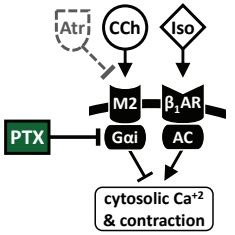


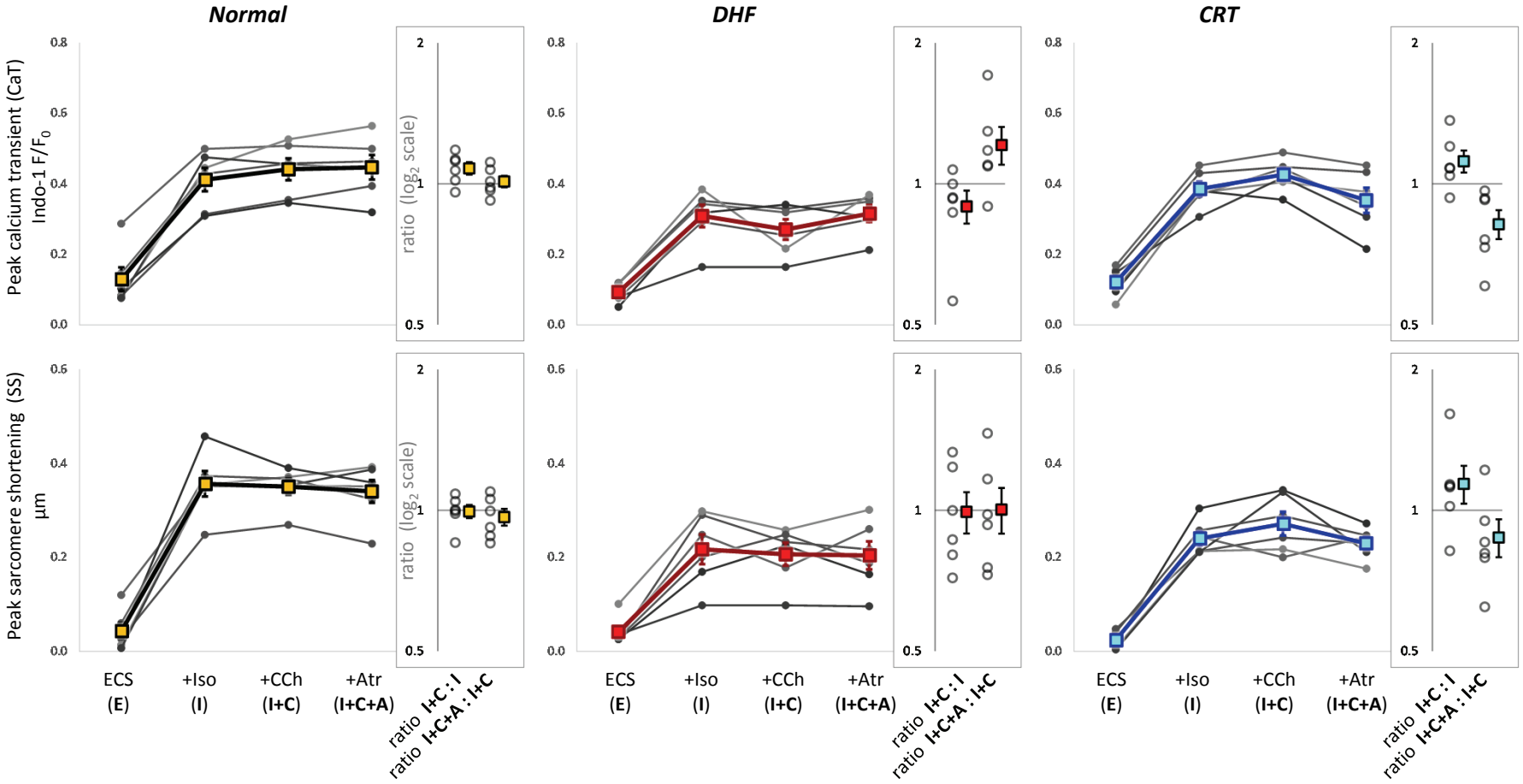
The 80% durations of the CaT (top row) and SS (bottom) are plotted in a format similar to that in Online Figure 4a.

For all groups, the C+A:C ratio was significantly different from the C:E ratio within each inset ($p<0.05$). Whereas the C+A:C ratio was larger than the C:E ratio in DHF, the converse was seen in normal and CRT ($p<0.05$).

ONLINE FIGURE V: Distinct effects of M2- and M3- muscarinic receptor signaling

a. Peak response to cholinergic stimulation in the presence of tonic β-adrenergic stimulation and PTX (Gαi inhibitor)



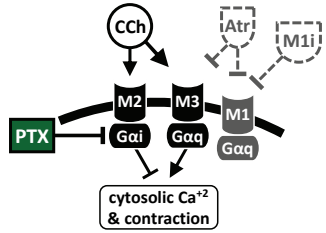


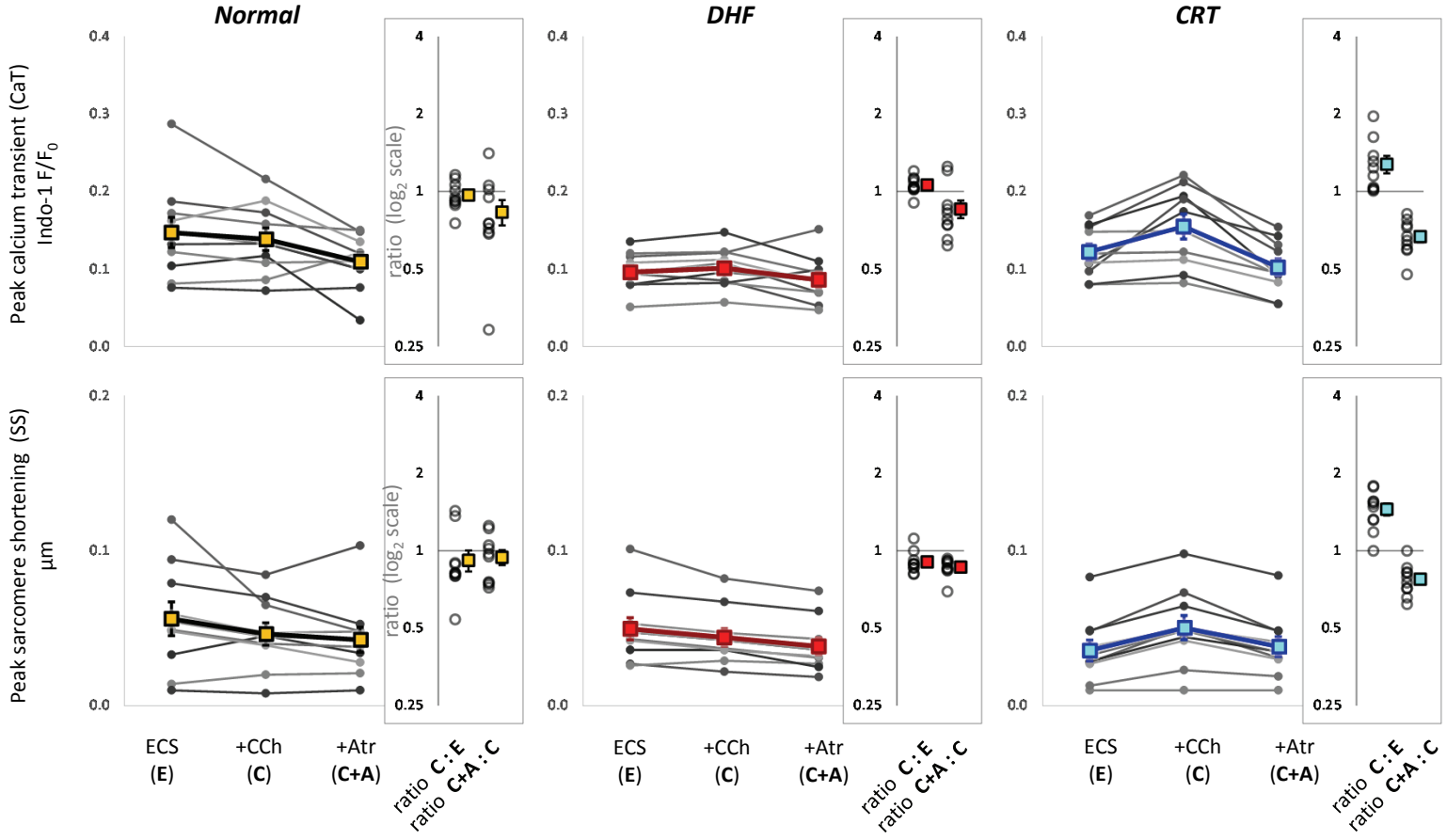
The 80% durations of the CaT (top row) and SS (bottom) are plotted in a format similar to that in Online Figure 2a.

Compared to the data in the absence of PTX (Online Fig. 2a), the corresponding I+C+A:I+C ratios and the I+C:I ratios were significantly diminished for all groups ($p<0.0001$).

ONLINE FIGURE V (continued):

b. Peak response to cholinergic stimulation alone in the presence of PTX (Gαi inhibitor)



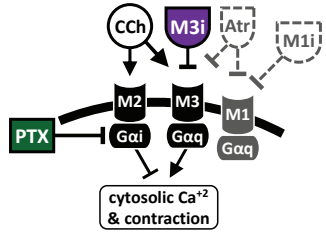


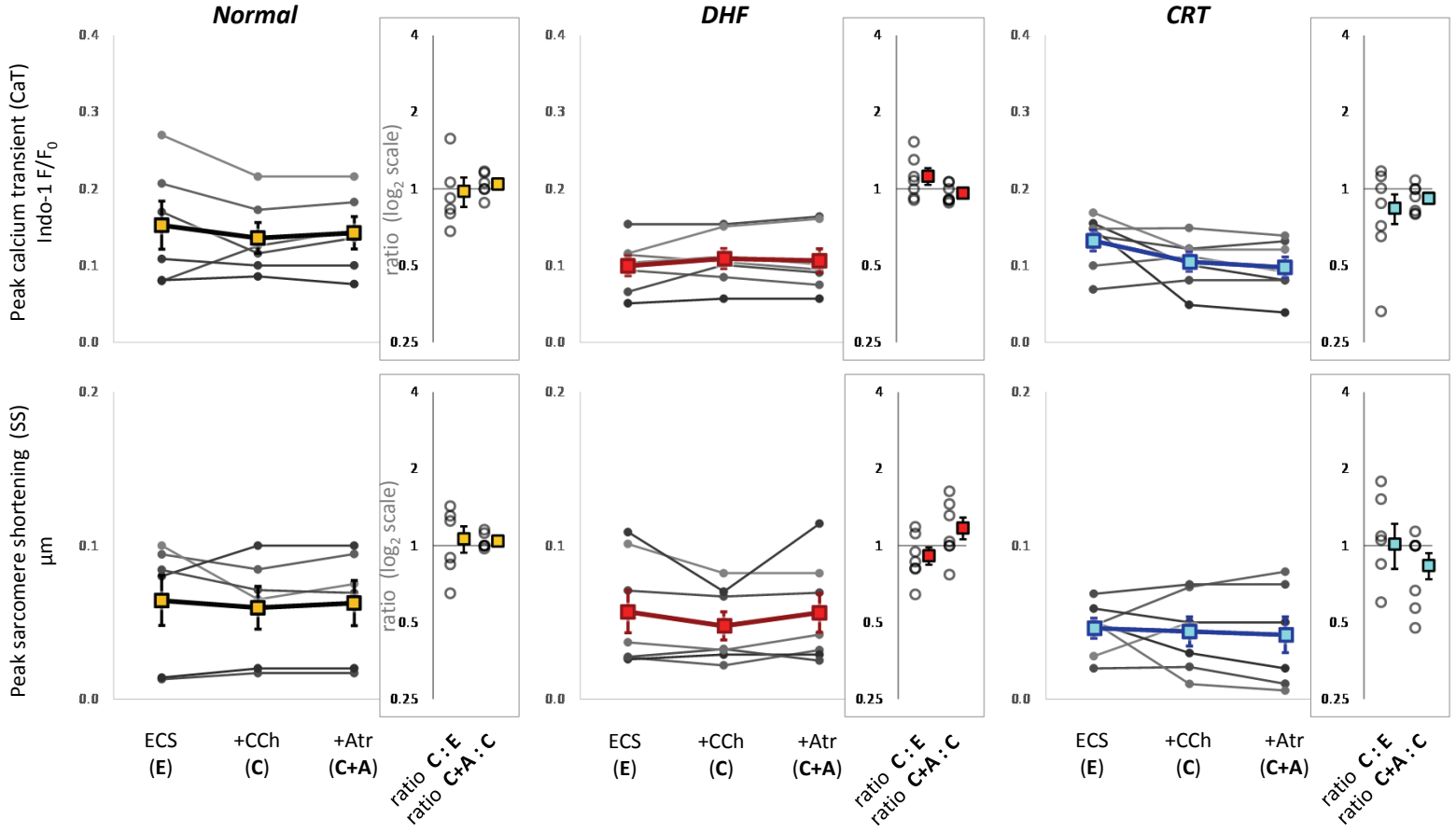
The peak CaT (top row) and SS (bottom) amplitudes in the presence of PTX and an M1-mAChR-specific inhibitor are plotted in a format similar to that in Online Figure 4a.

The C:E ratios for the CaT and SS in CRT were larger than those in normal and DHF ($p<0.05$), and the C+A:C ratios in CRT were smaller than those in normal and DHF ($p<0.05$).

ONLINE FIGURE V (continued):

c. Peak response to cholinergic stimulation alone in the presence of PTX (Gαi inhibitor) and M3-receptor inhibitor





The peak CaT (top row) and SS (bottom) amplitudes in the presence of PTX, an M1-mAChR-specific inhibitor and an M3-mAChR-specific inhibitor are plotted in a format similar to that in Online Figure 4a.

For all groups, the C+A:C ratio was similar to the C:E ratio within each inset. Compared to the absence of an M3-mAChR inhibitor (Online Fig. 5b), the corresponding C+A:C ratios for the CaT and SS were significantly different in CRT ($p<0.05$) but they were the same in DHF and normal.