The Rate- and Species-Dependence of Short-Term Memory in Cardiac Myocytes

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Abstract Short-term memory is an intrinsic property of paced cardiac myocytes that reflects the influence of pacing history, and not just the immediately preceding diastolic interval (DI), on the action potential duration (APD). Although it is recognized that short-term memory affects the dynamics of cardiac myocytes in general, and the onset of irregular cardiac rhythm in particular, its has never been adequately quantified or measured directly in experiments or numerical simulations, mainly due to the absence of appropriate techniques. As a result, very little is known about the rate- and species dependent behavior of short-term memory. In this study, we introduce a new approach that allows one to estimate how much short-term memory, M_S , is present in the cardiac myocyte at different pacing rates. The new quantification is based on the fact that pacing history affects not only the APD, but the entire dynamics of paced cardiac myocytes, in particular the restitution curve. Using the patch clamp technique and numerical simulations, we measured short-term memory restitution—the dependence of M_S on the cycle length—in isolated rabbit and guinea pig ventricular myocytes. In both species, M_S is rate- and species-dependent, displaying a biphasic behavior as a function of cycle length. Moreover, our results indicate that there is a significant difference in M_S measured between both species at small cycle lengths. Numerical simulations suggest that the kinetics of the rapidly activating delayed rectifier potassium current I_{Kr} is partially responsible for this difference.

Keywords Action potential duration · Short-term memory · Restitution · Cardiac myocytes

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1 Introduction

Short-term memory is an intrinsic property of paced cardiac myocytes that has been the subject of intense investigation in the field of electrophysiology during the past decade [1–5]. Although a clear definition of short-term memory has not been developed, it is generally accepted that short-term memory refers to the influence of pacing history, as opposed to the influence of the immediately preceding diastolic interval (DI), on action potential duration (APD). It is currently recognized that short-term memory plays an important role in the dynamics of cardiac myocytes in general, and the onset of irregular cardiac rhythms in particular [5–7]. For instance, the presence of short-term memory affects the slope of the restitution curve, which is the nonlinear functional relationship between the APD and the preceding DI. It has been proposed that the restitution curve is one of the major determinants of the dynamics of paced cardiac myocytes and its slope is thought to be related to the onset of life-threatening cardiac rhythms [8, 9].

However, despite its importance, until recently [10], short-term memory had never been quantified directly either in biological or numerical studies¹. This is mainly due to the difficulties associated with developing an appropriate quantification for the influence of pacing history on action potential dynamics. Typically, to study memory-related phenomena, the APD was assumed to depend on some artificial parameters representing the influence of pacing history. Consequently, various dynamical behaviors of such parameters have been proposed to mimic the experimental results [3–6]. Recently, it was demonstrated theoretically [11] that the introduction of such a parameter is equivalent to the assumption that APD depends on several preceding beats (APDs and/or DIs). It was then shown experimentally that up to three preceding beats [12, 13] play a substantial role in the control of the APD. However, this approach is somewhat artificial and does not allow an exact quantification of the short-term memory. Moreover, its validation by direct comparison with experiments is extremely difficult and has not yet been performed. As a result, very little is known about the dynamical behavior of short-term memory. For example, it is unclear if short-term memory displays rate- and/or species-dependent behavior. Furthermore, the ionic mechanisms underlying short-term memory are also unknown. To answer these questions, the current approach of measuring the influence of pacing history of the dynamics of paced cardiac myocytes has to be re-examined.

In the present study, we propose a novel quantifier of short-term memory based on the fact that, in the presence of short-term memory, the restitution curves and their slopes depend on the pacing protocol used to generate them [3, 7, 14, 15]. Specifically, we argue that the difference between the slopes of the dynamic and S1S2 restitution curves reflects the influence of pacing history, and therefore could be used to estimate the amount of short-term memory. We will call short-term memory measured in this way M_S , and we will call the dependence of M_S on pacing cycle length the *short-term memory restitution*. Our definition of short-term memory is unique in providing a numerical value for short-term memory at different pacing cycle lengths.

Using this definition, we measured short-term memory restitution in isolated rabbit and guinea pig ventricular myocytes, and compared the results with numerical simulations to study the rate- and species-dependent behavior of M_S . M_S was significantly different in the two species at high pacing rates. The numerical simulations suggested that the kinetics of the rapidly activating delayed rectifier potassium current I_{Kr} was partially responsible for this difference.

¹In [10], the memory amplitude of canine endocardium was calculated.

2 Materials and Methods

2.1 Solutions

The *standard Tyrode's solution* contained (mmol/l): NaCl, 148; CaCl₂, 1.8; KCl, 5.4; MgCl₂, 1.0; NaH₂PO₄, 0.4; glucose, 5.5; and HEPES, 15 (pH 7.4 adjusted with NaOH). This solution was used for cell isolation and also as the extracellular solution for action potential studies. The *pipette solution* for the whole-cell patch contained (mmol/l): K-aspartate, 90; KCl, 20; KH₂PO₄, 10; EGTA, 5; HEPES, 5; K₂ATP, 1.9.

For myocyte isolation, we used the following solutions: *Low Ca²⁺ Tyrode's solution* (mmol/l): NaCl, 148; KCl, 5.4; MgCl₂, 1; NaH₂PO₄, 0.4; glucose, 5.5; and HEPES, 15 (pH 7.2 adjusted with NaOH). One milligram per milliliter of bovine serum albumin was added to this solution. *Enzyme solution*: was identical to the low Ca²⁺ Tyrode's solution, with the addition of collagenase type II (100 units/ml for guinea pig, 200 units/ml for rabbit; Worthington Biochemicals, Lakewood, NJ). *KB (recovery) solution* (mmol/l): KCl, 80; MgSO₄, 5; KH₂PO₄, 30; glucose, 20; β -hydroxybutyric acid (sodium salt), 5; creatine, 5; ATP, 5; taurine, 20; EGTA, 0.25; pyruvic acid, 5; 25 mg/ml polyvinylpyrrolidone (PVP-40). *Cardioplegic solution* (mmol/l): glucose, 280; KCl, 13.44; NaHCO₃, 12.6; mannitol, 34.

2.2 Myocyte Isolation

Cardiac myocytes were isolated using the Langendorff retrograde perfusion method as previously described [16]. Adult animals of either sex (3 rabbits, 1.5–3 kg, 12 cells; 3 guinea pigs, 0.27–0.3 kg, 10 cells) were injected with heparin sulfate (300–500 U) and anesthetized with sodium pentobarbital (rabbits, 75 mg/kg i.v.; guinea pigs, 400 mg/kg i.p.). Hearts were quickly removed, immersed in cardioplegic solution and retrogradely perfused with Tyrode's solution (at 37°C) for ~8 min at 32 ml/min (rabbit) or ~5 min at 16 ml/min (guinea pig) to remove any excess blood in the vessels. The hearts were then perfused with Ca²⁺-free-Tyrode's solution for 10–12 min, followed by a 10-min (guinea-pigs) or a 40-min (rabbits) perfusion with the enzyme solution. Finally, the hearts were perfused with the KB solution. Subsequently, strips of tissue were carefully shaved off the left and right endocardial surfaces of each heart. The tissue strips from each ventricle were separated into tubes containing the KB solution and were mechanically agitated to free the myocytes. After a recovery period of ~30 min, the KB solution was gradually (~20 min) exchanged with normal Tyrode's solution. All experiments were performed at 37.7±0.5°C.

2.3 Electrophysiology

The whole-cell patch-clamp technique was used for current-clamp recordings. Borosilicate glass electrodes were pulled with a Flaming/Brown puller (model P-97), yielding a tip resistance of 3–5 M Ω when filled with pipette solution. Data were recorded with the use of an Axoclamp-2 A amplifier and the pClamp8 suite of programs (Axon Instruments, Union City, CA). Stimulus pulses were generated by PCI-6013 Basic Multifunctional I/O board (National Instruments, Austin, TX). A custom-written LabView program (National Instruments, Austin, TX) controlled stimulation. Action potentials were elicited by 5-ms square pulses at approximately twice the diastolic threshold amplitude. APD was measured at 90% of full repolarization. Action potential measurements were started about 2 min after patch rupture. Data were sampled at a rate of 20 kHz, filtered, and then stored on the hard disk of an IBM computer.

2.4 Numerical Simulations

The Luo and Rudy dynamic model (<http://www.cwru.edu/med/CBRTC/LRdOnline/>) and the Puglisi and Bers model [17] were used to conduct numerical simulations in guinea pig and rabbit cardiac ventricular myocytes, respectively. The following initial ionic concentrations were used (in mM): $[\text{Na}^+]_o = 140$, $[\text{K}^+]_i = 145$, $[\text{K}^+]_o = 5.4$, $[\text{Ca}^{2+}]_o = 1.8$, and resting $[\text{Ca}^{2+}]_i = 120$ nM, where “o” indicates extracellular and “i” indicates intracellular concentrations. APD was measured at 90% of full repolarization.

2.5 Pacing Protocol

The following pacing protocol [5] was used to obtain the restitution portraits [12] of paced cardiac myocytes and thus to visualize simultaneously the dynamic and the local S1S2 restitution curves at various cycle lengths S1:

- (1) 200 stimuli are applied at cycle length S1 to allow APD to achieve a steady state.
- (2) Ten stimuli are applied at the same cycle length S1 to measure the steady-state APD A^* .
- (3) One stimulus is applied at a longer interval $\text{S1S2} = \text{S1} + \delta$ ms to record APD after a long perturbation, A^+ .
- (4) Twenty stimuli are applied at cycle length S1 to achieve steady state after the long perturbation².
- (V) One stimulus is applied at a shorter interval $\text{S1S2} = \text{S1} - \delta$ ms to record APD after short perturbation, A^- .
- (VI) Twenty stimuli are applied at cycle length S1 to achieve steady state after the short perturbation².

The protocol was started at cycle length $\text{S1} = 1,000$ ms, and after completion of steps (I)–(VI) S1 was decreased by 100 ms, and steps (I)–(VI) were repeated. The value of δ was set to 50 ms (see Appendix). Figure 1a and b illustrate steps (II), (III) and (IV), (V), respectively, of the pacing protocol for $\text{S1} = 200$ ms. The DI was calculated as the difference between S1 or S1S2 and A^* . In standard usage, an S1S2 restitution curve refers to a plot of APD against δ that spans a range of values. Because we only probe two values of δ (short and long perturbations), we call our restitution curves ‘local’ restitution curves, to distinguish them from the full-blown restitution curves. The term restitution portrait, as coined by Kalb et al., [12] refers to a graph in which dynamic and S1S2 restitution curves [14] and APD accommodation [4] are superimposed. In this paper, we will refer to the graph with the former two curves as a restitution portrait.

2.6 Slope Measurement

The following procedures were used to calculate slopes of the dynamic restitution curve and the local S1S2 restitution curves in the restitution portrait at each cycle length S1:

Slopes of the dynamic restitution curve (S_{dyn}) The mean values of the steady-state responses A^* recorded during step (II) of the pacing protocol form the unique dynamic

²According to previous publications [12, 16, 20], 20 stimuli are enough to achieve steady state after long/short perturbations.

Fig. 1 Typical experimental traces of the action potentials recorded at $S1=200$ ms from isolated rabbit ventricular myocytes using the pacing protocol. The three last steady-state action potentials A^* , long perturbations A^+ (a) and short perturbations A^- (b) are shown. c Superposition of action potentials A^* , A^+ and A^- at $S1=500$ ms and $S1=200$ ms

restitution curve. These points were fitted with an exponential or polynomial function and S_{dyn} was calculated analytically at steady-state points for each $S1$.

Slopes of local S1S2 restitution curves (S_{12}) The responses from steps (II), (III) and (V) of the pacing protocol form the local S1S2 restitution curves at each value of $S1$. These responses were fitted with either a linear or quadratic function and S_{12} was calculated at the intersection with the dynamic restitution curve for each $S1$ (see [Appendix](#)).

Nonlinear curve fitting was performed using Origin 7.0 (Northampton, MA). Group data are presented as mean values $\pm$ standard error, where individual errors of slope measurements are also included. Statistical comparisons among groups were performed by ANOVA. $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 The Presence of Short-Term Memory in the Paced Cardiac Myocytes

Figure 1a and b illustrate typical experimental traces of action potentials obtained using the pacing protocol in isolated rabbit ventricular myocytes. In the Figure, the last three steady-

state action potentials (A^*) recorded at $S1=200$ ms are shown, together with the APD following the long (A^+ , $S1S2=250$ ms) and short (A^- , $S1S2=150$ ms) perturbations. Note that $A^+ > A^* > A^-$. The difference between A^+ , A^- and A^* depends on the $S1$ cycle length and is more pronounced as $S1$ cycle length decreases, as shown in Fig. 1c. Similar behavior was observed for isolated guinea pig ventricular myocytes and the corresponding guinea pig numerical simulations (data not shown).

Figure 2a shows a typical recording of a dynamic restitution curve (black) and local $S1S2$ restitution curves (gray) obtained from isolated rabbit ventricular myocytes, and Fig. 2d shows numerical simulations using rabbit ion channel kinetics. One can see from the figures that for each $S1$, the slope of the dynamic restitution curve S_{dyn} is clearly distinguishable from the slope of the local $S1S2$ restitution curve S_{12} , revealing the presence of short-term memory (see Fig. 2a, inset). Note that the results from the numerical simulations are qualitatively similar to those obtained experimentally. Although Figs. 2a and d describe the dynamics of the isolated rabbit myocytes, the guinea pig isolated myocytes demonstrate qualitatively similar behavior [16].

To estimate the influence of the short-term memory M_S on the dynamics of cardiac myocytes, we introduced the following quantification:

$$M_S = S_{\text{dyn}}|_{S1} - S_{12}|_{S1} = \alpha. \quad (1)$$

Thus, according to (1), M_S is equal to the difference between S_{dyn} and S_{12} measured at a given $S1$ (angle α in Fig. 2a). This definition reflects several experimental and theoretical facts demonstrating that in the absence of short-term memory, all restitution curves are identical [7, 18]. Equation (1) drastically differs from previous attempts to quantify short-term memory [3–5, 19] because it allows direct measurement of M_S restitution, both theoretically and experimentally. Note that, according to (1), M_S reflects the influence of pacing history not only on APDs, but on the restitution curves, which consist of both APDs and DIs. Specifically, M_S characterizes the disparity between restitution curves due to the influence of pacing history.

3.2 Rate- and Species-Dependence of M_S

To demonstrate the rate-dependence of M_S , we measured the S_{dyn} and S_{12} slopes at different values of cycle length $S1$ and then calculated M_S according to (1). The results are shown in Fig. 2b (patch clamp recordings) and Fig. 2e (numerical simulations) for isolated rabbit myocytes. Both S_{dyn} and S_{12} slopes increase monotonically as $S1$ decreases. In contrast, the rate-dependence of M_S is biphasic, with a maximum $S1$ around 600 ms in the rabbit experiments and 300 ms in rabbit simulations.

The comparison between the rate-dependence of M_S for the guinea pig and rabbit myocytes is shown in Fig. 2c (patch clamp recordings) and Fig. 2f (numerical simulations). As in rabbit, guinea pig's M_S was also a non-monotonic function of $S1$, but with a maximum at $S1$ of 400 ms (experiments) and 300 ms (numerical simulations). There was a statistically significant difference in the values of M_S (measured experimentally) at $S1 < 400$ ms; rabbit myocytes had significantly less memory than guinea pig myocytes ($P < 0.05$).

Note that the y-axis scales of Fig. 2a–c and d–f are different. Thus, there is qualitative, but not quantitative agreement between the experimental and numerical results. The APDs

Fig. 2 Results of patch clamp experiments (a–c) and numerical simulations (d–f). **a, d** Dynamic restitution curve (RC) and local S1S2 RC (gray) for different values of S1 obtained for isolated rabbit ventricular myocytes. The two intersecting curves in the *top left* corner of **a** shows a close-up view of the two restitution curves recorded at the same value of S1; the angle α is equal to M_S . **b, e** The rate-dependence of S_{dyn} , S_{12} and M_S calculated according to (1) for isolated rabbit ventricular myocytes. **c, f** The rate-dependence of M_S in the rabbit (black circles) and guinea pig (open circles) isolated myocytes. Note that M_S is significantly different in these species at small S1. Asterisks in **c** represent statistically significant data ($P < 0.05$)

calculated using numerical simulations are smaller at all pacing rates (for both species) which lead to shallower restitution curves and smaller values of M_S .

3.3 Insights into Ionic Mechanisms of M_S

The results from the previous section demonstrate that rabbit and guinea pigs myocytes have different values of M_S at high pacing rates. An important question that arises at this point is what ionic currents are responsible for species-dependent differences in short-term memory restitution. To answer this question, we used computer models of guinea pig (<http://www.cwru.edu/med/CBRTC/LRdOnline/>) and rabbit [17] ventricular myocytes.

It is known that the kinetics of various currents (I_{to} , $I_{Ca,T}$ and I_{Kr}) in these two models are different. Thus, to determine whether one of these currents was responsible for the species-dependence of M_S , we obtained the restitution portraits for the following three different cases: (1) the control rabbit model; (2) the control guinea pig model; (3) a rabbit model in which the kinetics for one of the currents were replaced by those from the guinea pig model. The values of M_S were calculated for different pacing frequencies for each case according to (1). The results are presented in Fig. 3, where the short-term memory restitution for the control rabbit (black circles) and guinea pig (open circles) simulations is shown in all three panels. The rabbit model with the kinetics of I_{Kr} and $I_{Ca,T}$ substituted by guinea pig kinetics are represented by stars in 3a and 3b, respectively. The rabbit model with no I_{to} is represented by stars in panel c³. Figure 3 shows that replacing rabbit I_{Kr} with guinea pig I_{Kr} brings M_S values at $S1 < 400$ ms much closer to guinea pig values, compared to substituting the guinea pig $I_{Ca,T}$ or excluding I_{to} . Therefore, the data suggests that the kinetics of I_{Kr} is responsible (at least partially) for establishing the species-dependent differences in the M_S at high frequencies.

4 Discussion

4.1 A New Quantification Method for Short-term Memory, M_S

The quantification for M_S presented in the paper is based on the fact that pacing history affects not only the APD, but also the entire dynamics of paced cardiac myocytes, in particular the restitution curve. Indeed, it is well known that, in the presence of short-term memory, the restitution curve depends on the pacing protocol used to obtain it [1, 2, 5, 10, 14]. Thus, we assumed that the difference between slopes of the restitution curves measured at the same cycle length using different pacing protocols would be proportional to M_S . This approach is going one step further than the existing approaches, in which the effect of pacing history is incorporated in APD only [1–5]. Instead, we claim that any memory-related changes of APD will change the dynamics of cardiac myocytes, causing the restitution curve to be dependent on the pacing protocols. Indeed, in the absence of short-term memory, the APD will depend only on the preceding DI, and, thus, the restitution curve will be unique [7, 8, 18]. Therefore, the actual difference between slopes of the restitution curves measured at the same cycle length using different pacing protocols is a direct consequence of the short-term memory.

³Note that guinea pigs do not have I_{to} .

Fig. 3 The influence of different ionic currents on the rate-dependence of M_S . The results of numerical simulation are shown for: guinea pig model (*open circles*); rabbit model (*filled circles*); **a** rabbit model in which the kinetics of I_{Kr} was taken from guinea pig model (*stars*); **b** rabbit model in which the kinetics of $I_{Ca,T}$ was taken from guinea pig model (*stars*); **c** rabbit model without I_{to} . Note that species-dependent differences in M_S at small S1 could be reproduced by changing the kinetics of I_{Kr}

It is worth mentioning that the quantification for M_S also takes into account the APD accommodation—another consequence of the short-term memory. This means that after abrupt change in the pacing rate, the APD needs time (up to several minutes) to achieve a new steady state [4, 12, 14, 16, 20]. The APD accommodation effect is included in (1) by assuming that steady state is achieved at each cycle length.

One of the limitations of the quantification for M_S (1) is that it might be protocol-dependent. Indeed, the values of M_S could be different if restitution curves are to be measured using different pacing protocols (for instance, dynamic and S1S2S3). However, to date, the restitution portrait is the only technique that allows measurements of various restitution curves (dynamic and local S1S2 restitution curves) at the same $S1$ without repeating the cycle length downsweep.

4.2 The M_S Restitution in Rabbit and Guinea Pig Ventricular Myocytes

The M_S restitution, i.e., the dependence of M_S on the cycle length, measured in rabbit and guinea pig ventricular myocytes using both experimental and numerical techniques, gives qualitatively similar results. As is expected, at large S1 (1,000 ms), both species have small values of M_S . This is due to the almost complete recovery of the ionic currents from inactivation and, thus, the negligible influence of pacing history on the APD and the restitution curves. As S1 decreases, (up to 500 ms), the value of M_S increases, reflecting the rising role of the pacing history. Interestingly, as S1 decreases further, the value of M_S decreases, indicating that restitution curves now depend less on the pacing protocol. Note that such biphasic dependence of M_S on the cycle length is shown for both experimental and numerical simulations. This result is in contrast to the previous data obtained using other ionic models, which demonstrated that the difference between the dynamic and the local S1S2 restitution curves is more pronounced at high pacing frequency [5, 7].

Our experimental and numerical results also demonstrate that M_S is significantly different at high pacing frequencies for rabbit and guinea pig myocytes. Numerical simulations suggest that the kinetics of I_{Kr} are at least partly responsible for such difference. The importance of I_{Kr} to the rate-dependent dynamics of cardiac myocytes and the onset of APD alternans was suggested previously [21, 22]. Taking this fact into account, it is possible that the value of M_S at the high pacing rate somehow reflects the species-dependent vulnerability to alternans. We are currently exploring this possibility.

4.3 Limitations of the Study

There are several limitations of our study that we would like to discuss. One of the major limitations of our study is the objective restriction connected with the use of ionic models for the description of the dynamics of periodically paced cardiac myocytes (<http://www.cwru.edu/med/CBRTC/LRdOnline/>), [17]. Although these models are based on real experimental data, they are unable to mimic the entire dynamical behavior of guinea pig and rabbit ventricular myocytes. This leads to the fact that the similarities between the experimental results and results of numerical simulations are qualitative, not quantitative.

The second limitation of this study is the presence of EGTA (5 mM) in the pipette solution. It is known that EGTA clamps the intracellular calcium concentration and thus affects a number of cellular mechanisms including calcium-dependent inactivation of the L-type calcium current and the sodium-calcium exchanger. This might change the dynamics of cardiac myocytes, especially, at high pacing rate. In particular, the incidence of alternans is higher if intracellular calcium cycling is not clamped [23]. On the other hand, the influence of EGTA on the steady-state restitution curve is not significant (see, for instance, Fig. 5a of [15], and [23]). Indeed, we previously showed that the restitution curves obtained using perforated patch (minimum distortion of intracellular calcium compartment) and ruptured patch (EGTA=5 mM) techniques were almost identical [15]. Based on these conflicting results, it is difficult to predict the exact effect of intracellular calcium cycling on the results presented in the manuscript. Thus even though the present results suggest that I_{Kr} plays a role in short-term memory in the normal system, calcium currents could be involved as well, and may have an even stronger contribution to memory.

Another limitation of our study is the dependence of the S_{12} slope on the magnitude of the long and short perturbations, δ . Our numerical simulation results (presented in the [Appendix](#)) suggested that $30 \text{ ms} < \delta < 70 \text{ ms}$ introduces $\delta < 70 \text{ ms}$ minimal disturbance to the M_S measurements. Although we do not have enough data to determine the range of δ experimentally, we believe that it should be similar. Indeed, if δ is small enough, the calculation of S_{12} slope would be difficult, due to large noise in the experimental set up. On the other hand, large δ (at short perturbations) will lead to the loss of 1:1 capture.

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Appendix

The Influence of δ on S_{12} Slope

According to the pacing protocol, three points (obtained at steady-state conditions, and long and short perturbations) are used to calculate the slopes of local S1S2 restitution curves, S_{12} . However, the magnitude of the long and short perturbations δ (steps III and V of the pacing protocol) could affect the slope measurements. Figure 4a shows the restitution portraits obtained from numerical simulations of the rabbit model for different values of δ : 10, 20, 30, 50, 70 and 100 ms. One can see from the Figure that the dynamic restitution curves (black circles) are superimposed and thus do not depend on δ (since steady state has been reached). Note that the local S1S2 restitution curves (open circles) are almost linear at larger S1 and their curvature increases as S1 decreases. Thus the value of the perturbation δ will be more important at high frequencies. To estimate this effect, we performed the following procedure at each value of cycle lengths: we calculated S_{12} using (1) three-points-fit for different values of δ (S_{12}^δ) and (2) full-fit using all values of δ (S_{12}^{full}). Then, we defined the error of S_{12} measurements for each value of δ as

$$\text{error} = \frac{S_{12}^\delta - S_{12}^{\text{full}}}{S_{12}^{\text{full}}} \quad (2)$$

Figure 4b illustrates the magnitude of error as a function of δ for different values of S1: 200, 300 and 600 ms. Recall that the curve for S1=600 ms is a representative curve for all values of S1>300 ms. As expected, the smaller the S1, the larger the error for all values of δ . In addition, Fig. 4b demonstrates that the error is larger for both small and large values of δ . Note that large δ at short perturbations might lead to a loss of 1:1 capture at high pacing rates. Figure 4c shows the dependence of M_S (or α) on δ for different values of S1. Overall, the results demonstrate that the magnitude of δ within the range between 30 ms to 70 ms induces minimal disturbance to the slopes and short-term memory measurements. Note that M_S strongly depends on δ only for S1=200 ms. In the manuscript, we choose $\delta=50$ ms.

Fig. 4 **a** Dynamic (black circles) and local S1S2 (open circles) restitution curves obtained for different values of δ . The error of S_{12} measurements calculated according to (2) **(b)** and M_S (or α) **(c)** as a function of δ for different values of S1: 200 ms (triangles), 300 ms (circles), 600 ms (squares)

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