Optical Mapping Analysis of the Spatiotemporal Pattern of Experimental Tachyarrhythmia in Improved Isolated Rat Atrium Preparation

Tetsuro SAKAI
Department of Physiology, University of the Ryukyus School of Medicine, Okinawa, 903-0215 Japan

Abstract: We have studied the experimental tachyarrhythmia in an improved isolated rat atrial preparation for the optical mapping of excitation spread. The atrial preparation, including the right or left auricle, was dissected from the adult rat heart, and an artificial hole was made in the center of the preparation. The preparation was then stained with a fast merocyanine-rhodamine voltage-sensitive dye (NK2761). Using a multi-element (16 × 16) photodiode array, the spread of excitation was assessed optically by timing the initiation of the action potential–related extrinsic absorption changes. In comparison with the intact isolated right atrial preparation, which we used previously, the mapping of the excitation spread was much easier and more precise because of the simple structure of the preparation. The electrical stimulation applied by a bipolar electrode evoked the sustained excitation with a fast rhythm, which we termed “experimental tachyarrhythmia” (ET). We optically mapped the spatiotemporal patterns of the spread of excitation during the initiation and the maintenance phases of ET. In most cases, a rotation of the excitatory wave around the artificial hole, i.e., a circus movement of the excitatory wave, was observed. These maps suggest that this circus movement resembles the basic mechanism of the tachycardia-like excitation observed in the intact isolated right atrial preparation. On the other hand, the appearance of an ectopic pacemaker with a fast rhythm was also observed. In some examples, two ectopic pacemakers appeared simultaneously. We consider that the experiment using the improved preparation is a superb in vitro model of atrial arrhythmia.

Key words: rat atrium, voltage-sensitive dye, multiple-site optical recording, circus movement, ectopic pacemaker.

To elucidate the basic mechanism(s) of atrial arrhythmia, we have been carrying out experiments using optical methods for monitoring membrane potential activity using fast voltage-sensitive dyes [1, 2]. We optically mapped the spread of excitation in the intact isolated rat right atrial preparation during an abnormal state of atrial rhythm, which we termed “tachycardia-like excitation”, evoked by electrical stimulation [3–5]. During this event, the occurrence of the excitatory waves shows a much faster rate than that in the normal condition, and the physiological pacemaker does not work. From the optical mapping, we demonstrated that the reentry mechanism took part in tachycardia-like excitation. In most cases, the excitatory wave rotated around an anatomical obstacle (mostly the ostium of the superior vena cava). Moreover, in some examples, abnormal automatism, i.e., an ectopic pacemaker, also took part in the generation of tachycardia-like excitation. This phenomenon is interesting because transient complex patterns of excitation spread were observed during the initiation phase of the event before the establishment of the stable reentry pattern. Furthermore, event-to-event variations were observed in the excitation spread pattern. This phenomenon seems to be an in vitro model of atrial tachycardia or atrial flutter [4].

Throughout these studies, we found serious disadvantages in the use of intact atrial preparations. The intact right atrial preparation has some anatomical structures, such as the junction of the atrial septum and left atrial free wall, that make thicker parts in the preparation. These parts lead to the loss of incident light, resulting in a low S/N ratio of optical signals. This problem makes the lacking parts of the map, often prevents the perfect mapping of the excitation spread, and decreases the yield of experiments using intact atrial preparations.

In the present work, to investigate the generality of the arrhythmogenic mechanisms observed in tachycardia-like excitation more clearly, we have developed an improved preparation made of the auricle part of the right or left atrium, which has a relatively simple structure, to avoid this disadvantage. These preparations have no thicker portions with a fast rhythm, which we termed “experimental tachyarrhythmia” (ET). We optically mapped the spatiotemporal patterns of the spread of excitation during the initiation and the maintenance phases of ET. In most cases, a rotation of the excitatory wave around the artificial hole, i.e., a circus movement of the excitatory wave, was observed. These maps suggest that this circus movement resembles the basic mechanism of the tachycardia-like excitation observed in the intact isolated right atrial preparation. On the other hand, the appearance of an ectopic pacemaker with a fast rhythm was also observed. In some examples, two ectopic pacemakers appeared simultaneously. We consider that the experiment using the improved preparation is a superb in vitro model of atrial arrhythmia.

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Correspondence should be addressed to: T. Sakai, Department of Physiology, University of the Ryukyus School of Medicine, 207 Uehara, Nishihara, Okinawa, 903-0215 Japan. Phone: +81-98-895-1111, Fax: +81-98-895-1403, E-mail: tsakai@med.u-ryukyu.ac.jp

to cause a loss of incident light. Furthermore, we made an artificial hole in the preparation because during the tachycardia-like excitation evoked in intact right atrial preparations, the excitatory wave often rotated around the natural hole of the preparation, i.e., the ostium of the superior vena cava. We applied electrical stimulation to this preparation to evoke a sustained excitation with a fast rhythm, which we termed “experimental tachyarrhythmia” (ET) (to distinguish it from “tachycardia-like excitation” in the “intact” right atrial preparation). In this way we could map the spread of excitatory waves during the initiation and maintenance phases of ET, using a voltage-sensitive merocyanine-rhodanine dye together with a 16 × 16–element photodiode array. These experiments proved that the tachyarrhythmia, which has properties similar to the tachycardia-like excitation in the intact preparation, could be evoked in this improved preparation. This is an enumerative expansion of our studies of experimental atrial arrhythmia. We consider that this preparation could be a more simple and superb in vitro model of atrial tachyarrhythmia.

Preliminary reports of this work have appeared in abstract form [6].

MATERIALS AND METHODS

Preparations. This study was approved by the Animal Care and Use Committee, University of the Ryukyus, and was conducted in accordance with its recommendations. Adult rats (Wistar strain, 200–500 g) of both sexes were anesthetized by the inhalation of ether. The hearts were quickly removed and bathed in an ice-cold bathing solution. The right or left auricle preparation was incised. As shown in Fig. 1, an artificial hole was made in the center of the preparation. Note that the thickness of the preparation is almost regular and no thicker portions were observed. The preparations were attached, positioning the endocardial side upward, to the silicone (KE106LTV, Shin-etsu Chemical Co., Tokyo, Japan) bottom of a simple chamber by pinning with tungsten wires. The preparations did not include the physiological pacemaker, and most of them exhibited no spontaneous excitation. The preparations were kept in an oxygen-equilibrated bathing solution with the following composition (in mM): NaCl, 149; KCl, 5.4; CaCl$_2$, 1.8; MgCl$_2$, 0.5; Tris HCl buffer (pH 7.4), 10; and glucose, 10. For the suppression of optical artifacts resulting from contractile movements, 2,3-butanedione monoxime (BDM: 10–20 mM) was added to the bathing solution. In the previous reports [3–5], no systematic changes were observed in the conduction of excitation in BDM-containing solution.

Staining. The isolated atrial preparations were stained for 30 min in a bathing solution containing 0.5 mg/ml of a fast voltage-sensitive merocyanine-rhodanine dye (NK2761, Hayashibara Biochemical Laboratories Inc.) [7]. The dye was first dissolved with a small amount of dimethyl sulfoxide (DMSO) for dispersion, and the bathing solution was then added. The final concentration of DMSO was 0.25% v/v. After being stained, the preparations were washed with several changes of normal bathing solution containing BDM. Neither phototoxic effects nor pharmacological actions could be observed during the use of this dye, which had a fairly long bleaching time [1–4, 7].

Optical measurement. An optical recording system, designed to record the absorption change of NK2761 as an action potential–related optical signal, equipped with a 16 × 16–element photodiode array was used [4]. In this system, the preparation chamber was mounted on the stage of a microscope (Type FLUOPHOTO-VFD, Nikon Inc., Tokyo, Japan). Light from a 300 W tungsten-halogen lamp (Type JC-24V/300W, Kondo Sylvania Ltd., Tokyo, Japan) driven by a stable DC power supply (Model NL035-20, Takasago Ltd., Kawasaki, Japan) was collimated, rendered quasimonochromatic with an interference filter having a transmission maximum of 701 ± 11 nm (Asahi Spectra Co. Tokyo, Japan), and focused on the preparation by means of a bright field condenser. The objective (×1) and the relay lens (×1) projected a real image of the preparation onto a 16 × 16–element silicon photodiode matrix array (C4675, Hamamatsu Photonics Ltd., Hamamatsu, Japan). Each pixel (element) of the array detected light transmitted by a square region (620 × 620 μm$^2$) of the preparation. The output of each detector in the...
diode array was fed into a PC-based recording system (Model ARGUS-50/PDA, Hamamatsu Photonics Ltd.). The acquisition rate was 0.5 or 2 ms/frame. Since the built-in software package in this recording system was designed for experiments of neurophysiology, several additional programs written originally by the author were used to display and analyze the data obtained from cardiac preparations. Using this optical recording system, we were able to simultaneously measure the cellular electrical activity from 256 contiguous areas in the preparation. All the optical signals shown in this paper were obtained in a single sweep. Optical recordings were carried out in a still chamber without continuous perfusion with the bathing solution at a room temperature of 23\degree–27\degree C. The incident light was turned off to avoid bleaching of the dye, except during the measurement period.

The contraction-related artifact could be discriminated from the action potential–related signals by its wavelength dependence: the action potential–related signals were completely eliminated at 620 nm, the null wavelength of NK2761, whereas the contraction-related artifacts remained at 620–630 nm [1–3]. The use of this wavelength enabled the contraction-related artifacts to be easily discriminated from the action potential–related optical signals.

RESULTS

Circus movement around the artificial hole

Experimental tachyarrhythmia (ET) was evoked by electrical stimulation applied with a bipolar electrode placed on the preparation. Figure 2A illustrates an example of the optical recording of the initiation and maintenance of ET evoked by a short train of current pulses applied with a bipolar electrode (about 3 diastolic threshold intensity, duration 10 ms, 5 Hz, 5 shocks). Action potential–related optical signals were recorded simultaneously from the isolated atrial preparation with an artificial hole using the 16 \times 16–element photodiode array, and optical signals recorded by 4 elements are displayed. A sketch of the preparation imaged on the photodiode array and the positions of the individual elements in the matrix array are shown on the right. In the beginning of the record, optical action potentials evoked by the stimulation were observed. Just after the end of stimulation, rhythmical excitation appeared with an interval of about 400 ms. Although the repolarization phases of the optical action potentials were distorted by the contraction-related artifact (indicated by filled circles) in several traces, optical signals indicated that the membrane potential repolarized to the resting potential level, and then the next action potentials occurred. Note that the contraction-related artifact appeared only in the repolarization phase of the optical action potential, and that the upstroke phase (indicated by arrowheads) of the action potential was not distorted by it. During ET, the amplitude of the optical action potential was almost the same as that of the optical action potential evoked by stimulation. This event was sustained for about 10 min.

In Fig. 2B, the optical action potentials during ET are displayed in a fast sweep speed. As can be seen in this record, the optical action potential appeared in the order of traces d, c, b, a, and again d. This indicates that the excitatory wave rotated around the artificial hole in a counterclockwise direction. We measured the timing of the feet of optical action potentials in the whole recording area and constructed maps of the propagation of the excitatory waves.

The maps of excitation spread in the initiation and early maintenance phases of ET shown in Fig. 2 are represented in Fig. 3. Six maps made over time are displayed. The po-
The positions of the wave fronts are displayed as isochrone curves with intervals of 50 ms. In the first map (0–250 ms), the excitation evoked by the first stimulation appears at the left edge of the recording area (indicated by an open triangle) at 88 ms and propagates in two ways, i.e., the upper route and the lower route, around the hole to the right area. In the second and third maps (250–550 ms and 450–750 ms), the excitatory waves are evoked at 288 ms and 488 ms and propagate by two routes, as in the first map. Note that in these maps, the intervals of the isochrone curves are narrower than in the first one, indicating a slowing of the conduction velocity. In the fourth map (600–900 ms), the excitatory waves are evoked at 688 ms and propagate to the right and along the lower route to the right lower area. The appearance of this blocked area is the start of the circus movement of the excitatory wave around the artificial hole.

Event-to-event variation

In Figs. 4 and 5, the initiation processes of ET evoked in a preparation are shown. These events were evoked by 3 shocks at 5 Hz. In Fig. 4A, the optical signals during the initiation and early maintenance phases of the first ET event are shown. After the establishment of ET, as indicated by gray arrows, the optical action potentials appeared in the order of a, b, c, d, and again a, indicating that the excitatory wave rotated around the artificial hole in a counterclockwise direction.

The initiation process of this event is shown in Fig. 4B. In the first and second maps, the excitatory waves evoked by stimulation at 42 ms and 238 ms propagate to the right and left areas, respectively. In the third and fourth maps, the excitatory waves propagate along the upper route and collide with the fourth excitatory wave in the right upper area. The excitatory wave propagating along the lower route begins to rotate around the artificial hole, as shown in the sixth map (1,000–1,450 ms). This is the process of the initiation of the circus movement of the excitatory wave around the hole.

Fig. 3. Optical mapping of the initiation of ET. Sequential maps during the initiation of ET (Fig. 2) are shown. The positions of the wave fronts are displayed as isochrone curves at 50 ms intervals. In the fifth and sixth maps, the wave front starts at the thick isochrone curves labeled 900 (fifth map) and 1,000 (sixth map). The gray arrows indicate the pathway of the spread of the excitatory wave. The gray line labeled “b” in the fourth map is the blocked area over which the excitation could not propagate. The central gray areas are the areas where the optical signal could not be detected because of the artificial hole. The peripheral gray areas are the areas where the delay could not be measured because of noise, artifacts, or absence of preparation. The triangles at the upper left edge of the first five maps indicate the areas where the action potential was first generated by the stimulation.
area along the upper and lower routes. In the third map, the upper route is obstructed by the blocked area (indicated by "b"), and the excitatory wave propagating along the lower route starts to rotate around the hole in a counter-clockwise direction. This event continued for 4 min.

After the spontaneous end of the ET event shown in Fig. 4, another event was evoked. The optical signals during the initiation and early maintenance phases of the second event are shown in Fig. 5A. After the establishment of ET, as indicated by gray arrows, the optical action potentials appear in the order of d, c, b, a, and again a, indicating that the excitatory wave rotated clockwise around the hole. This pattern continued for more than 10 min. Note that the events shown in Figs. 4 and 5 are examples of event-to-event variation in the ET. As shown in Table 1, the circus movement of the excitatory wave around the hole is a major pattern of ET.

Circus movement with double wave fronts

In Fig. 6, another rare type of circus movement of excitatory waves is seen. As shown in Fig. 6A, during the ET event the optical action potentials appeared in the order of a, b, c, d, and again a, indicating that the excitatory wave ro-

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**Fig. 4.** Optical mapping of the event-to-event variation of ET (1). A: Optical signals during the initiation and early maintenance period of ET. Optical action potentials detected simultaneously from four different positions (a–d) are shown. The time course of the electrical pulse (about 3 diastolic threshold intensity, 1 ms, 5 Hz, 3 shocks) applied with a bipolar electrode placed on the preparation is shown by arrows below the traces (Stim.). The optical action potentials appeared in the order of a, b, c, d, and again a, indicating that the excitatory wave rotated counterclockwise around the artificial hole. B: Optical mapping of the initiation of ET. Sequential maps during the initiation of ET shown in A are presented. Other conventions as in Figs. 2 and 3.
tated around the hole in a clockwise direction. In the pairs of traces, a and c and b and d, optical action potentials appeared nearly simultaneously, indicating that two excitatory waves rotated simultaneously around the hole. The maps of the spread of excitation are shown in Fig. 6B. Two wave fronts are clearly observed, and this event continued for more than 10 min. This type of circus movement is a rare pattern: only two cases were observed (see Table 1).

**Ectopic pacemaker**

Another mechanism of ET was observed in some preparations. Figure 7 shows the initiation and early maintenance phases of the ET event observed in the same preparation as that shown in Fig. 2. Although the event was evoked by the same stimulation procedure as for Fig. 2, the spread pattern of the excitatory wave was quite different. The maps of spread patterns are shown in Fig. 8. In the first to the fifth maps, the excitatory waves evoked by stimulations propagate to the right area along the upper and lower routes. After stimulation ended, ectopic pacemakers (indicated by "P") appear in the upper left edge.

**Table 1. Incidence of the patterns of experimental tachycardia in the auricle preparation with an artificial hole.**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Number of preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circus movement</td>
<td>8</td>
</tr>
<tr>
<td>Simple</td>
<td></td>
</tr>
<tr>
<td>Double wave fronts</td>
<td>2</td>
</tr>
<tr>
<td>Ectopic pacemaker(s)</td>
<td>5</td>
</tr>
<tr>
<td>Single</td>
<td>3</td>
</tr>
<tr>
<td>Double</td>
<td>2</td>
</tr>
<tr>
<td>Failed to evoke tachycardia</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
</tr>
</tbody>
</table>

(1) One preparation exhibited four different patterns of tachycardia.
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Fig. 6. Circus movement with double wave fronts. A: Optical action potentials detected simultaneously from four different positions (a–d) are shown. The optical action potentials appeared in the order of a, b, c, d, and again a, indicating that the excitatory wave rotated around the artificial hole in the clockwise direction. In the pairs of traces a and c and b and d, the action potentials appeared nearly simultaneously, indicating that two excitatory waves rotated around the hole. B: Optical mapping of the circus movement with double wave fronts. Two wave fronts rotated around the hole in a clockwise direction. Other conventions as in Figs. 2 and 3.

Fig. 7. Optical action potentials during the initiation and early maintenance periods of ET. Optical action potentials detected simultaneously from four different positions (a–d) are shown. This record was obtained from the same preparation as in Figs. 2 and 3. The pattern of circus movement was not observed. Other conventions as in Fig. 2.

Incidence of the patterns of ET

The incidence of the excitation spread patterns of ET is summarized in Table 1. The major pattern of ET was the simple circus movement around the artificial hole. The circus movement with double wave fronts was a rare pat-
The incidence of ectopic pacemaker(s) was relatively low. Systematic differences were not observed between the preparations made of the right atria and those of the left ones. Note that one preparation exhibited four different patterns. The event-to-event variation was not a rare phenomenon.

**DISCUSSION**

In this study, we have enumeratively expanded our optical study on atrial arrhythmia. To evaluate the generality of the mechanisms of the generation of the atrial arrhythmia observed in tachycardia-like excitation, we have developed improved preparations made of the auricle part of the right or left atrium, which has a relatively simple structure, with an artificial hole. In comparison with the “intact” right atrial preparation, the thickness of the improved preparation is thin and more regular. In the optical recording, this property suppresses the variation of DC-background intensity of the incident light, which causes an overflow of the photodetector because of too much high background intensity and/or a reduction of S/N ratio because of low background intensity. This property is the strong merit of using this preparation in an optical mapping study. Actually, when “intact” preparations were used, pixels lacking the data were inevitable in all preparations. These pixels were much fewer in the map of the improved preparation. The quality of the excitation spread maps is much better in this improved preparation. This is the most distinct methodological improvement in the present study.

We were careful in interpreting the maps, since the structure of the improved preparation is artificial. We always compared the results obtained in the improved preparation with the phenomena observed in the intact right atrial preparation. Fortunately, the obtained experimental results in this study were not so complex because of the simple structure of the preparation, and we did not find serious experimental drawbacks in using the improved preparation.

We optically analyzed the spatiotemporal pattern of the excitation spread during the ET event in this improved preparation. The above experimental results of the electrical activity of the isolated rat atrial preparation during the optically monitored ET using a fast voltage-sensitive dye provide the basic nature of this phenomenon. As seen in Table 1, the ET event is mainly based on the stable circus
movement of excitatory waves (i.e., “macro reentry”) around the artificial hole. This is the most important characteristic of ET. This pattern of reentry was first described by Mines [8] as “closed-circuit reentry” [9]. In the previous study using “intact” isolated right atrium preparations [4], we demonstrated this type of circus movement during tachycardia-like excitation. ET and tachycardia-like excitation share a common mechanism.

As can be seen in Figs. 3, 4B, and 5B, transient blocked area(s) appeared during the initiation phase of the circus movement of excitatory waves. The appearance of a conduction block is the start of the circus movement. In the previous work [4], in “intact” right atrium preparations, a transient complex pattern of excitation spread (including unstable blocked areas) was observed during the initiation phase of the tachycardia-like excitation. The initiation of ET is much simpler than that of tachycardia-like excitation. It is plausible that the simplicity of the initiation of ET is due to the simplicity of the anatomical structure of the improved preparation.

As shown in Figs. 4 and 5, the appearance of the transient blocked area is not fixed. The irregularity of the appearance of this area leads to an event-to-event variation of ET. As also reported the event-to-event variation of the conduction pattern of the excitatory waves during the events of tachycardia-like excitation in “intact” atrial preparation [4]. This irregularity of the blocked area would also happen in “intact” preparations.

The mechanisms for the generation of the conduction block are still unknown. We suggested in a previous work [4] that because of repetitive excitation, the intracellular accumulation of Ca\(^{2+}\) causes an increase in the resistance of the gap junction channel [10], which results in a block of the excitation spread. The intracellular Ca\(^{2+}\) dynamic in atrial preparations remains totally unsolved. An intracellular Ca\(^{2+}\)-imaging study would be helpful to solve this problem.

The circus movement of excitatory waves with double wave fronts shown in Fig. 6 is the most interesting new finding in this study. We have never observed this type of circus movement in intact right atrial preparations. Although the mechanism of the separation of the wave fronts and the condition for the appearance of this phenomenon are still unknown, the size of the artificial hole may contribute to its appearance. The relatively large size of the hole in comparison with the ostium of the superior vena cava, which is the common center of circus movement in the intact preparation, makes the length of the reentry circuit longer. This long path would be favorable for a circus movement with double wave fronts.

The contribution of an ectopic pacemaker(s) is another feature of ET. The ectopic pacemaker was also observed in the “intact” right atrium preparation during the tachycardia-like excitation [4]. Furthermore, as shown in Fig. 9, double pacemakers were observed during the ET event in the improved preparation. Note that double pacemakers were not observed in the intact right atrium preparation. Although the cellular mechanism(s) of the generation of ectopic pacemaker(s) is unknown, it is very interesting that the same procedure (i.e., electrical stimulation) induced the appearance of a transient blocked area, which results in the circus movement of the excitatory wave, and
also the ectopic pacemaker. It is suggested that some common mechanisms may induce the blocked area and ectopic automatism. In addition, slow diastolic depolarization was not observed in the ectopic pacemaking area in this study (data not shown).

The event-to-event variation shows one of the important characteristics of ET as discussed earlier. Since applications of the same electrical stimulation did not evoke the same results, there is no reproducibility in the strict meaning in this experiment: ET is not a deterministic phenomenon, but a chaotic event. This suggests that during the initiation phase of ET, the atrial cells in the preparation were functionally unstable and the electrical connections between cells (i.e., gap junctions) became loose, allowing abnormal automatism to occur easily. In this condition, small fluctuations in the process of the generation of ET resulted in the irregular appearance of the blocked area(s) and/or ectopic pacemaker(s), which led to the event-to-event variations. Unfortunately we have no idea about how to describe this phenomenon quantitatively: these problems will be addressed in future experiments.

The comparison of tachycardia-like excitation in the intact preparation with ET in the improved preparation is summarized in Table 2.

Using Langendorff-perfused rat heart preparations together with optical recording techniques, Hayashi et al. evoked atrial arrhythmias by burst pacing stimulation [11]. They reported two types of atrial arrhythmia. One was “atrial tachycardia” in which periodic single wave fronts of activation appeared, and the other was “atrial fibrillation”, in which 2 to 4 independent wave fronts propagated in different directions. The former seems to be the same phenomenon as the one we reported as tachycardia-like excitation and ET, though they did not show the complete map of excitation spread. We have not observed the atrial fibrillation-like phenomenon in the “intact” atrial preparation and in the improved isolated rat atrium preparation with an artificial hole. Nor did we observe such a phenomenon in the isolated rat atrial preparation in the intact heart. We consider that ET is an in vitro model of atrial tachycardia or atrial flutter. Further studies using this system with pharmacological and/or clinical electrophysiological approaches would be beneficial.

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Table 2. Comparison of tachycardia-like excitation in the intact preparation and ET in the improved preparation

<table>
<thead>
<tr>
<th>Thickness of the preparation</th>
<th>Relatively irregular</th>
<th>Relatively regular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reentry around the anatomical obstacle</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Reentry without anatomical obstacle</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Reentry with double wave fronts</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Abnormal automatism</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Double pacemakers</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Event-to-event variations</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

1) Refs. 3–5.

REFERENCES

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