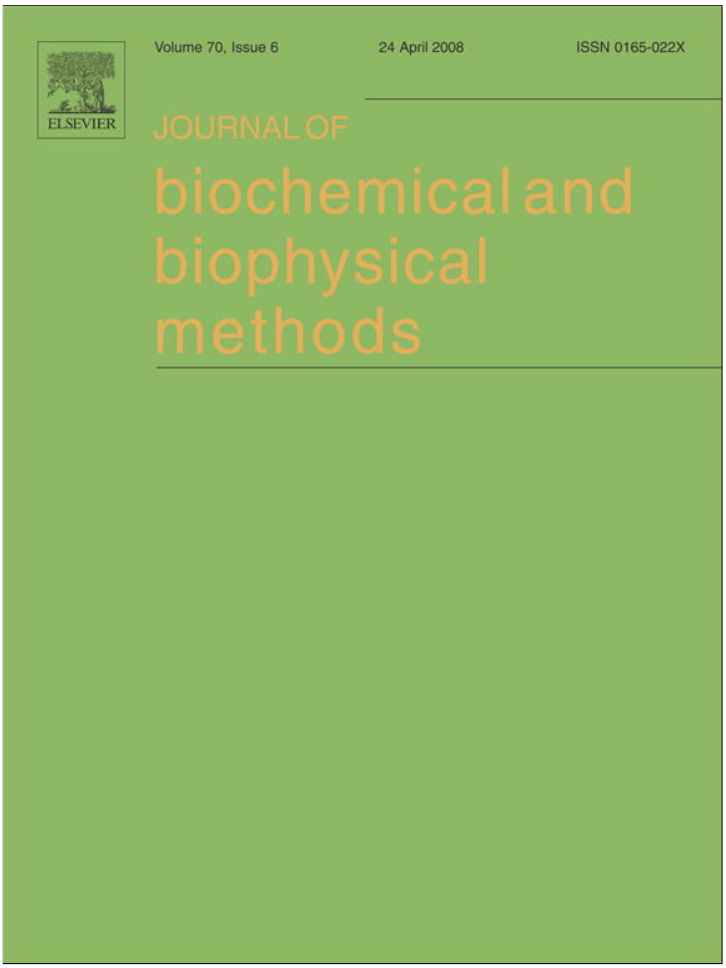

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A rapid and non leaky way for preparation of the sharp intracellular recording microelectrodes

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Abstract

To fill microelectrodes using backfilling method needs excessive time approximately 4–6 h. It is often difficult to fill microelectrodes without damage or leakage. A main problem is bubble formation in microelectrodes which has an impact on the electrical properties of the electrode and thus it influences the quality of the recording. Based on Archimede's principle there is a force within a solution which pushes insoluble material with a lower specific gravity upward and outside of the solution. Centrifugation can increase the force to eliminate the bubbles.

We designed a microelectrode holder to protect microelectrode sensitive tips from mechanical damage due to the gravity tensions; it can help to eliminate the bubbles easily and simultaneously in 10 min or less.

The tests were performed for 2000, 4000, and 8000 rpm centrifugation each one for 3, 6 and 12 min duration respectively, it was found that the bubbles were completely eliminated at 8000 rpm for 6–12 min and there were no significant differences for resistance, and the number of leaky or damaged electrodes between the two methods.

In the new design of devices, the materials used and the design of the holder are simple and the approach is applicable to many laboratories worldwide.

Keywords: Bubbles elimination; Intracellular recording; Glass sharp microelectrode

1. Introduction

Sharp glass microelectrodes for intracellular recordings in small cells, e.g. hippocampus pyramidal cells, have a high resistance of around 70 to 100 MΩ when filled with 3 M KCl.

Their shanks are very long and the diameter of their tips is less than 0.1 μm [1,2], in spite of the capillary action as a result of the microfilament existing within the microelectrode, filling a sharp glass microelectrode, without any damage, leakage or air bubbles getting into it, by using the usual backfilling method, is often difficult and consumes considerable time around 4–6 h for the sharp microelectrodes. It can be one of the important factors that restrict intracellular recording, especially when it is considered that because of the possible leakage or existence of the tiny bubbles in the shanks, near the tip, many electrodes after filling are not useable. In addition, sometimes, during finding the new suitable cell for recording, filled electrode has to be replaced with another one because of unsuitable resistance or tip cloggier. Preparation and replacement of the new electrodes may then takes longer time and may lead to undesired changes in the environment of the cells within the slice, so making it unsuitable for recording and wastes the time, animals, materials and efforts. Thus having a stock of well prepared microelectrodes during each recording session or experiment is very useful and important. In this report we describe a rapid and non leaky way for eliminating the tiny bubbles getting inside the intracellular recordings sharp glass microelectrodes.

Based on Archimedes' principle, within a fluid which contains insoluble material with lower specific gravity, e.g. bubbles inside

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the micropipette solution; a buoyant force acts to push them upward and outside the solution [3]. Centrifugation can increase this force depending on the relative centrifugal force or its rate and can remove the bubbles out. This is the principle which governs the way presented here for eliminating the bubbles inside the microelectrodes and preparing them accurately; without damage or leakage.

Cerf and Cerf also had used centrifugation with their special holder. Their holder helps them to use the centrifuge to force the fluid into the micro tip. Therefore they filled microelectrodes that didn't contain microfilament by forcing the fluid into the micro tip using centrifugation [4], thus the principle which governs their method for filling the electrode is different from the method we present here for eliminating the bubbles inside the micro tip. In our method the force inside and outside the microelectrodes are equal all the time and there is no penetration of the fluid toward the micro needle tip. It seems that the Cerf and Cerf method is abandoned after employment of microfilament within the microelectrodes because of a logical disadvantages. In their method; because of centrifugation; the force inside the micro tip would be higher than that of the outside; it would be a reason which increases the probability of micro needle leakage. Their method is quitted today and the time problem for filling of the sharp intracellular recording microelectrode using usual back filling method still persists. So the technical note which is presented here can solve the problem without production of damaged or leaky microelectrode.

2. Materials and methods

2.1. How to fill the microelectrodes

Microelectrodes (Borosilicate glass capillaries with filament, OD = 1.2 and ID = 0.49 mm) are pulled by a horizontal micropipette puller (APP-H Horizontal Micropipette Puller, 52500V, Stoelting, USA) for intracellular recording as long, thin and sharp micro needles. Electrodes are filled with the electrolyte solution (3 M KCl) through gentle and exact insertion of the solution inside and near the electrode shoulder by a fine poly ethylene tube [PE 10, OD = 0.61 and ID = 0.28 mm] connected to a syringe with a needle gauge 29. The presence of the bubbles inside the microelectrode is disregarded. As it is shown in Fig. 1A in the first step, a sampler tip A [above wide aperture OD = 8.1 and ID = 5.1 mm with 49 mm length] that is previously filled by electrolyte solution is inserted within another sampler tip (B) [like A] which its tip is previously closed by a paste [white tag] or the flame of a cigarette lighter and filled by electrolyte solution. In order to prevent from the microelectrode tip damage, two tip samplers must be selected between the different manufactured types as tip to tip distance should be more than 17 mm when the sampler tip A is fitted within the sampler tip B. As it is shown in Fig. 1B, in the second step, at a horizontal position the microelectrode is inserted within the sampler tip A until its trunk tangents the inside wall of the sampler tip, it is then gently rotated to a vertical position in order to make the microelectrode slide down within the sampler tip A to reach and pass its narrower end and grips and mounts there because of the bigger microelectrode shoulder diameter. Bubbles which come out during centrifugation must be replaced with solution. Thus it is important that all the length of microelectrode would be under the solution surface in the sampler tip A. Sampler tip A holds the microelectrode. Filled sampler tip B protects the highly sensitive microelectrode tip from any physical contact and during centrifugation against relative centrifuge force and the leakage possibility. The tip of sampler tip B must be closed to prevent the solution efflux during centrifugation, thus the pressure within the microelectrode solution and the sampler tip B would be equal all the time during the centrifugation. Therefore no solution efflux would happen from the microelectrode tip.

At the third step (Fig. 1C) this collection is inserted within a centrifuge special tube and the tube aperture is closed by Parafilm. It can help the investigator to maintain an ample of well prepared microelectrodes for some hours without any solution evaporation and crystallization within the microelectrodes. It is possible to make such collection as many as our centrifuge rotor capacity contains. In the fourth step (Fig. 1D) the final collection is inserted easily within the centrifuge tube holder in the rotor which its radius is 16 cm here and then the centrifuge is rotated at 8000 rpm up to 12 min maximum.

Relative centrifugal force (RCF) is expressed in units of gravity (times gravity or $\times g$). The relationship between centrifuge speed (revolutions per minute, rpm) and RCF is as follows:

$$g = (1.118 \times 10^{-5})RS^2$$

where g is the relative centrifugal force, R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in rpm [3]. Here, centrifugation of a sample at 8000 rpm in a centrifuge with a rotor radius of 16 cm will release a centrifugal force of 11,448.32 $\times g$.

Now the microelectrode within each collection is ready to use or can be stored in the laboratory for many hours until it is used.

To take the electrode, first the Parafilm cap should be removed from the centrifuge tube. Then by rotating the tube, the collection and sampler tips can now be taken out, and then by using a fine forceps, the end part of the pipette, which can be seen from the wide aperture of the sampler tip A, can be reached and caught; the electrode can now be gently pulled out.

2.2. Evaluation tests and finding the suitable rate and time duration of centrifugation

We prepared 10 groups each with 16 sharp microelectrodes. The mean length of microelectrode shanks were 14.5 ± 1.39 mm, microelectrodes of one group were filled by the back filling method for comparison. The microelectrodes of the other 9 groups were then centrifuged at different times and rates. Segurita centrifuge type BHG 1100 was used with centrifuge rotor capacity of 16 and centrifuge rotor radius equal to 16 cm.

Tests were performed at 2000, 4000 and 8000 rpm, each for 3, 6 and 12 min respectively.

In similar conditions, the important factor which plays the main role to produce undesirable resistance is the bubble

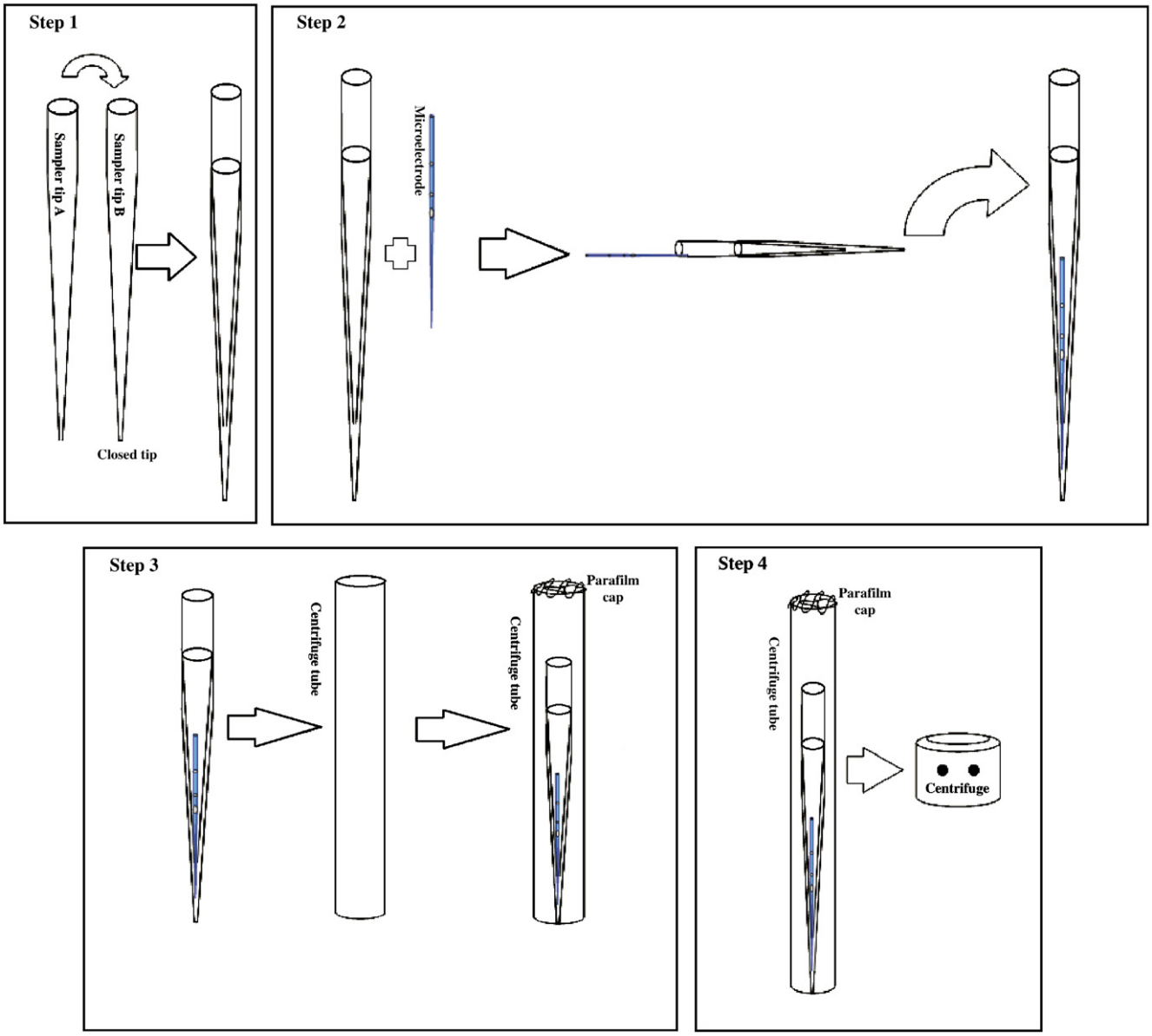

Fig. 1. A) Step 1, a sampler tip A filled by electrolyte solution is inserted into another sampler tip B with its tip closed and filled by electrolyte solution. For prevention of micro needle damage; tip to tip distance should be more than 17 mm when tip A is inserted and fitted within tip B. B) Step 2, in horizontal position, the microelectrode is inserted into the sampler tip A. It is then gently rotated to vertical position in order to make the microelectrode slide down within the tip sampler to reach its narrower end C) Step 3, the sampler tip with the microelectrode inside is inserted within a centrifuge tube and its aperture is closed by Parafilm. D) Step 4, the centrifuge tube is then rotated at 8000 rpm for 12 min inside a centrifuge.

formation within the electrodes shanks, thus removal of the bubbles without any damage to electrodes was considered as a criterion for the improvement of electrode preparation by the above mentioned method. Bubbles within the shank, from shoulder to tip of the microelectrodes, were counted using a usual photomicroscope at magnification of 10×40 respectively. Damaged or leaky electrodes and those with unsuitable resistance were counted at the end of procedure.

In another test, living purkingi cell within the cerebellum slices of male normal wistar *Rat* (70–90 g) was used to compare the quality of intracellular recording for new method of the microelectrode preparation, presented here, with usual backfilling method. All experiments followed the guidelines for animal care approved by committee for scientific ethics, Tarbiat Modares University.

2.3. Statistics

The results are expressed as mean ± SEM. The difference between groups over the centrifugation time course and rate of the centrifugation determined by two-way analysis of variance (ANOVA), followed by the Tukey's test with 5% level of significance.

For the electrode quality comparison between usual back-filling method and the method presented in this article, the statistical test of quality, chi-square (χ^2), was used.

3. Results

3.1. Results for evaluation tests

Tests were performed for 2000, 4000, and 8000 rpm each one at 3, 6 and 12 min. duration times respectively. The results show that the bubbles decreasing by centrifugation are time and rate dependent. As the Fig. 2 shows the bubbles are limited to zero at 8000 rpm for 6 to 12 min. As Table 1 shows the quality and electrode properties in 8000 rpm at 3 to 12 min centrifugation is equal to those with back filling method with regard to resistance and leaky or damaged sharp microelectrodes.

In contrast to usual backfilling method, there is no significant difference between the traces recorded from purkingi cell in the slices of the *Rat* cerebellum for new method of preparation of intracellular recording microelectrode (Fig. 3).

4. Discussion

There are different types of glass microelectrodes in electrophysiology which are related to the types of recording, e.g. extra or intracellular recording, thus there are different ways to fill them with electrolyte solution. Insertion of a small bundle of glass fibers in the tubing before being drawn out enable the fluid to flow from the shaft by capillary action and reach the micro tip [5]. So using the glass capillaries with already fixed filament made microelectrode filling easier and faster [6,7]. Now it seems that back filling method is the most common way in the laboratories for intracellular recording microelectrode preparation. However, it is relatively difficult and requires much time because sharp microelectrodes have longer shank and more resistance [8] and filling them should be performed without pressure to prevent leakage.

Measurement of the microelectrode resistance is possible just after filling them, and in most of the instances, microelectrodes are used only once because of their tips sensitivity and high probability of blockage or leakage after penetrating the electrode into the tissue or cell. Thus, several microelectrodes are required during single recording session. Without ample supply of well prepared electrodes, the time spent in filling them may lead to loss of cell or tissue for good recording and pose restriction in this type of investigations. The method we have described is fast, easy and accurate for removing the bubbles getting inside of several intracellular glass microelectrodes simultaneously, without any damage or leakage.

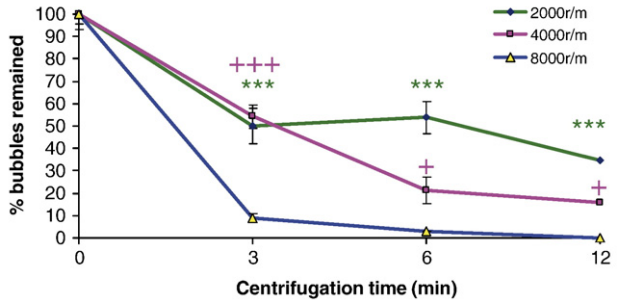

Fig. 2. Tests were performed for 2000, 4000, and 8000 rpm each one at 3, 6 and 12 min duration times respectively. The bubbles decreasing by centrifugation are time and rate dependent (ANOVA, $f_{(4,\ 131)}=3.5$, $p<0.0095$). As shown in the diagram, the bubbles are limited to zero at 8000 rpm for 6 to 12 min without any significant damages (Data as mean±SEM, $n>14$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significant difference between group 2000 rpm and 8000 rpm in the same time, $+p<0.05$, $++p<0.01$, $+++p<0.001$ significant difference between group 4000 rpm and 8000 rpm in the same time, Mean of microelectrodes shank length=14.5±1.39 mm).

Table 1
Number of leaky or damaged sharp microelectrodes and electrodes with suitable and undesirable resistance in both the back filling and the centrifugation bubbles out method at different rate and time

		Leaky and Damaged	Suitable resistance (40–70 MΩ)	Undesired resistance	Chi-square test results (χ^2)
Back filling		3	12	1	–
Centrifuge at 2000 rpm	3 min	1	4	11	106.66
	6 min	2	6	8	52.33
	12 min	4	5	7	40.41
Centrifuge at 4000 rpm	3 min	0	5	11	107.08
	6 min	2	5	9	101.08
	12 min	4	8	4	10.66
Centrifuge at 8000 rpm	3 min	2	11	3	4.13
	6 min	3	12	1	0
	12 min	2	13	1	0.413

Chi-square test results are shown for quality control compared to the back-filling method. The difference between expected values (the ratio of different electrodes in back filling group) and observed values (the ratio of different electrodes in each groups of centrifugation) are significant with $p>0.05$ when $\chi^2>5.99$, $n=16$, $df=2$.

In the above method, sampler tip A is used to hold micro-electrode and filled sampler tip B is used to protect the highly sensitive microelectrode tip from any physical contact. It also protect against relative centrifuge force and possible leakage making during centrifugation.

Cerf and Cerf had made a specific holder and employed the centrifugation to fill the microelectrodes at the time that the fixed microfilament was not invented to facilitate the filling of the glass electrodes [4]. They made the holder just to hold the electrode from its shoulder in the centrifuge, but the tip of the electrode is surrounded only by air, so during centrifugation the solution pressure within the electrode shank becomes very high and it helps the solution to penetrate and pass through the microelectrode tip. After the application of the fixed microfil-ament within the microelectrodes, their method is abandoned because of the increasing force inside the micro needle that logically leads to make them leaky. In contrast to the Cerf and Cerf method, there isn't any passage of fluid from the micro-electrode tip in our method, because of the tip closure in sampler tip B that is filled by solution, thus the pressure outside and inside the tip is equal so the bubbles come out from the wide aperture of the microelectrode because of the increasing solu-tion force toward the bubbles to push out and eliminate them during centrifugation. The advantages here is that all the time during centrifugation the forces inside and outside the micro-electrode tip wall are equal so it can prevent the sensitive tip from any damage or leakage.

Sampler tip A must be filled with solution and the length of electrodes should be within fluid range and be placed under the

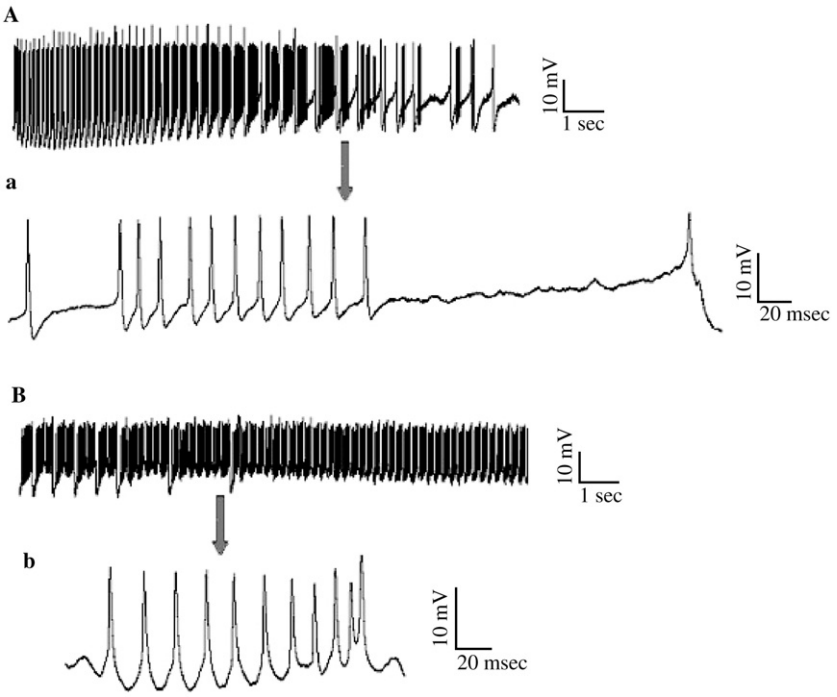

Fig. 3. A) Burst activity recorded by the electrodes filed using usual backfilling method a) action potentials recorded in an expanded burst by the electrodes filed using backfilling method. B) Burst activity recorded by new method presented in the text b) action potentials recorded in an expanded burst by new method. In contrast to usual backfilling method, there is no significant difference in the quality of recording, from purkingi cell in the slices of the *Rat* cerebellum, for new method.

solution surface, for the replacement of solution with the bubbles that come out during centrifugation.

Parafilm cover is used to make the maintenance of the filled microelectrode possible for a longer time, without any evaporation of solution that may lead to alteration in the concentration of electrolytes or crystallization.

There are many parameters that play important role in the resistance or impedance of microelectrodes. This includes the microelectrode shank length, the tip aperture diameter or electrolyte component, but at similar conditions the most important factor which plays the main role in producing undesirable resistance during filling of the electrodes is bubbles formation within the electrodes shanks. So in this study the removal of the bubbles was considered as a criterion for the improvement of filling by the mentioned method.

There isn't any quality difference between microelectrodes filled by back filling or those filled via centrifugation at 8000 rpm at 3 to 12 min since their resistance and the number of the damaged and leaky electrodes (Table 1) and the quality of recording from the living cells (Fig. 3) are equal. The most important advantages of this method are saving time and convenience.

In conclusion, our data shows that this technical note can help us to prepare the microelectrode in a short time by an accurate manner. Intracellular recording sessions would be easier and less time consuming using this note and it can provide power facilitation for intracellular recording investigation.

5. Simplified description of the method and its applications

Microelectrodes are filled with electrolyte solution through insertion of the solution near the electrode shoulder by a fine poly ethylene tube connected to a syringe. A sampler tip (A) previously filled by electrolyte is inserted within another previously filled one (B) with its tip closed. At a horizontal position the microelectrode is inserted within the sampler tip, and then all of the collection is gently rotated to vertical position in order to make the microelectrode slide down within the tip sampler A to reach and pass its narrower end and grips there. All the length of microelectrode must be placed under the solution surface. Then it is inserted within a centrifuge special tube and the tube aperture is closed by Parafilm. Final collection is placed within the centrifuge rotor and rotated at 8000 rpm up to 12 min maximum. Now the microelectrode within each collection is ready to use. To take the electrode, by using a fine forceps, the end part of the pipette, which can be seen from the wide aperture of the sampler tip A, can be reached and caught; the electrode can then be gently pulled out.

This technical note can help us to prepare the microelectrode in a short time with an accurate manner. Intracellular recording sessions would be easier and less time consuming using this note and it can provide power facilitation for intracellular investigation.

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