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Multi-Unit and Multi-Path system of the neural network can explain the steep dose-response of MAC

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Abstract
Purpose. The slope of the dose-response curves of inhalation anesthetics is steep around the minimum alveolar concentration of inhalation anesthetics (MAC) value. Contrastingly, the anesthetic dose-response curves of ion channels and enzymes are gradual. This discrepancy in the steepness may be a key to solve the mechanisms of anesthesia. To explain the steepness we propose a mathematical model of the neural network related to MAC.
Methods. We assumed that, in order to show movement in response to a noxious stimulus, a signal needed to be transmitted from A to B. There are m conduction pathways (Multi-Path) in the nerve network between A and B, and there are n conduction units (Multi-Unit) in each conduction pathway. Anesthetics bind to each conduction unit and block signal transmission. Anesthetics prevent movement in response to a stimulus, when at least one conduction unit among all conduction pathways has been blocked. We derived the equation for the probability of the signal being blocked by anesthetics.
Results. The steep dose-response curve of in vivo anesthesia requires a very large number of conduction units ($n > 100$) and conduction pathways ($m > 10^6$). The EC_{50} for each conduction unit was at least 3.8-fold larger than the apparent EC_{50} for the whole system under the experimental condition of simulation.
Conclusions. We constructed a model for the neural networks that relates to MAC as a Multi-Unit and Multi-Path system (MUMPS). To obtain highly cooperative dose-response curves comparable to those of in vivo anesthesia, at least 10^6 conduction pathways and more than 100 conduction units are required for each pathway. In these systems, the apparent anesthetic potency on the whole system (MAC) is much stronger than the anesthetic action on each unit. Because of this discrepancy, it is important to set anesthetic concentrations appropriately for experiments with in vitro systems.

Key words Dose-response curve · Anesthesia mechanism · MAC

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Introduction

The MAC (minimum alveolar concentration of inhalation anesthetics) is recognized as the standard measure of anesthetic potency, because immobilization by inhalation anesthetics to noxious stimuli is the most desirable and obvious endpoint of anesthesia [1,2]. Anesthesia mechanisms have been extensively studied using physical chemistry, molecular modeling, physiology, pharmacology, and whole-animal experiments [2].

The slope of the dose-response curves of inhalation anesthetics is steep around the MAC value. These steep dose-response curves are analyzed by the logistic plot, and the slope is calculated by the Waud equation [3]. The slope values of clinical anesthesia are often expressed by the Hill coefficient (n_H), and are in two-digit values ranging from about 6 to 20 [4]. The Hill equation is a convenient method to determine the steep dose-response curve, but it is often misused. The steep dose-response curves guarantee that, in clinical anesthesia practice, almost all patients become immobilized. Contrastingly, the anesthetic dose-response curves of ion channels and enzyes are gradual, and the n_H ~ value is usually less than 3 [4–6].

The discrepancy in the steepness between whole-animal and in vivo study systems may be due to the lack of a method for functional analysis of the neural network, and such a method will be a key to solve the missing information on anesthesia mechanisms. The present communication deals with the derivation of a mathematical model of the neural network, and examines the effect of an anesthetic on this model.

Methods

We propose the following model for the neural network between points A and B. It is assumed that there are m conduction pathways (Multi-Path) in the network, and

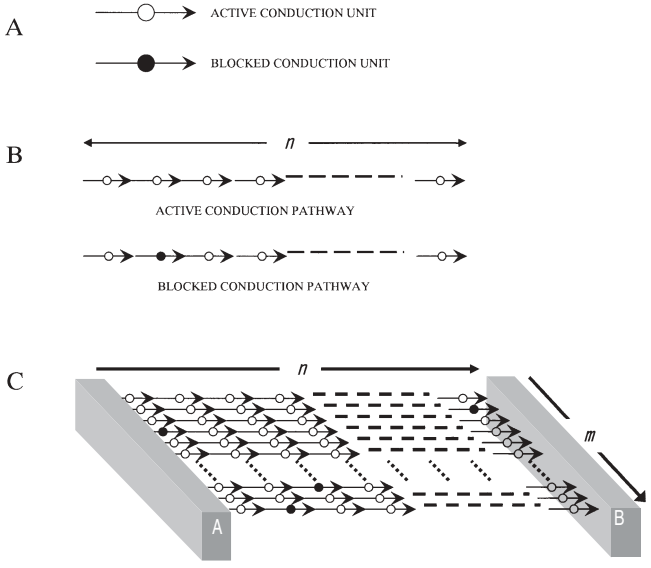

Fig. 1A–C. Multi-Unit and Multi-Path system (MUMPS). **A** The conduction unit: the *open circle* is the conduction unit, which can transmit the signal, and the *closed circle* is the unit blocked by anesthetics. **B** The conduction pathway consists of *n* conduction units. When one of the conduction units in each conduction pathway is interrupted, the conduction by that pathway is blocked. **C** A schematic presentation of the network, which consists of *m* conduction pathways. The signal is transmitted between points A and B when the conduction is maintained in at least one among *m* conduction pathways. Immobilization ensues when the conduction is totally shutdown

n conduction units (Multi-Unit) in each conduction pathway. Patients do not respond to noxious stimuli when the conduction between A and B is lost.

Figure 1 shows a schematic presentation of the proposed mathematical model. Anesthetics affect each conduction unit. When an anesthetic molecule binds to that conduction unit, conduction by the unit is interrupted (Fig. 1A). When one of the *n* conduction units in each conduction pathway is interrupted, the conduction by that pathway is blocked (Fig. 1B). Additionally, we assume that the patient responds to noxious stimuli when the conduction is maintained at least in one among *m* conduction pathways. Immobilization ensues when the conduction is totally shutdown (Fig. 1C).

An equilibrium constant K determines the possibility of inducing anesthesia.

$$E + A \leftrightarrow R$$
$$K = \frac{[R]}{[E] \times [A]} \tag{1}$$

where A is the anesthetic, E is the awake state, and R is the resting state (R state, unable to conduct) of the conduction unit.

The probability p of each conduction unit being in the R state is:

$$p = \frac{[R]}{[E] + [R]} = \frac{K[A]}{1 + K[A]} = \frac{[A]}{EC_{50}^{unit} + [A]} \tag{2}$$

where EC_{50}^{unit} is the anesthetic concentration that inhibits 50% of the conduction unit.

The probability that the i-th pathway fails to conduct, P_i, is:

$$P_i = 1 - (1 - p)^n. \tag{3}$$

Therefore, the probability, Y, that all of the conduction roots are incapable of signal conduction (immobilized) is:

$$Y = \left\{1 - (1 - p)^n\right\}^m = \left\{1 - \left(\frac{EC_{50}^{unit}}{EC_{50}^{unit} + [A]}\right)^n\right\}^m \tag{4}$$

In this highly complicated system with many conduction units and pathways, there is a possibility that the anesthetic potency on each conduction unit (EC_{50}^{unit}) may not match the apparent anesthetic potency on the total system (EC_{50}^{system}). Equation 5 is obtained by setting $Y = 1/2$ and $[A] = EC_{50}^{system}$:

$$EC_{50}^{system} = \left\{\frac{1}{\left(1 - 0.5^{\frac{1}{m}}\right)^{\frac{1}{n}}} - 1\right\} \times EC_{50}^{unit} \tag{5}$$

To evaluate the steepness of the dose-response curve, we also calculated the ratio of EC_{50}^{system} and EC_{95}^{system} (effective concentration of 95%):

$$EC_{95}^{system} / EC_{50}^{system} = \frac{\left\{\frac{1}{\left(1 - 0.95^{\frac{1}{m}}\right)^{\frac{1}{n}}} - 1\right\}}{\left\{\frac{1}{\left(1 - 0.5^{\frac{1}{m}}\right)^{\frac{1}{n}}} - 1\right\}} \tag{6}$$

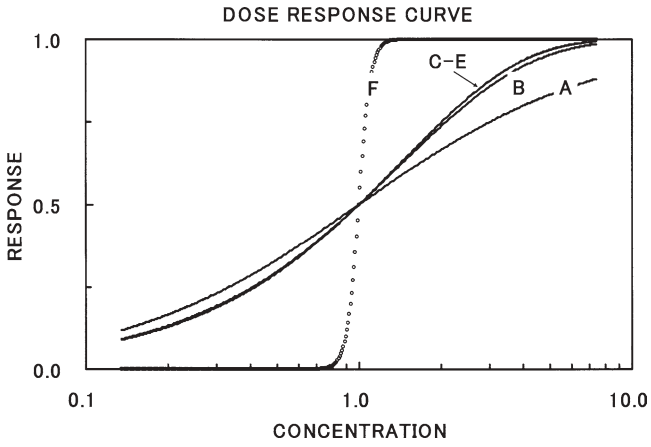

Fig. 2. Effects of the number of conduction units (n) on dose-response curve in MUMPS model with a single conduction pathway ($m = 1$). Curves *A–E* were calculated from Eq. 4, with *A*, $n = 1$; *B*, $n = 10^1$; *C*, $n = 10^2$; *D*, $n = 10^4$; and *E*, $n = 10^6$. *Open circles* (*F*) represent the dose-response curve calculated from Hill's equation, with a Hill number of 20

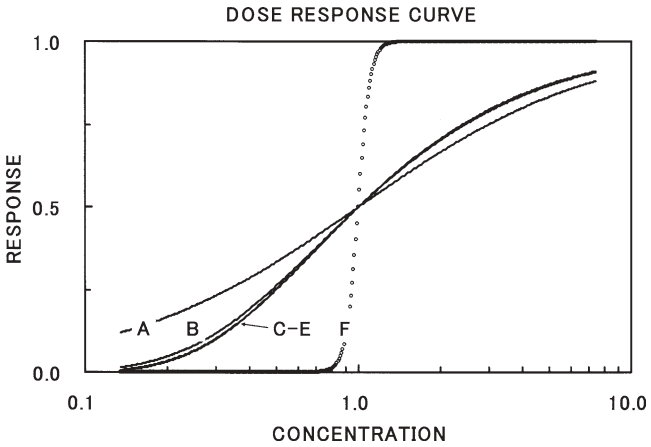

Fig. 3. Effects of the number of conduction pathways (m) on dose-response curve in MUMPS model with a single conduction unit ($n = 1$). *Curves A–E* were calculated from Eq. 4, with *A*, $m = 1$; *B*, $m = 10^1$; *C*, $m = 10^2$; *D*, $m = 10^4$; *E*, $m = 10^6$. *Open circles* (*F*) represent the dose-response curve calculated from Hill's equation, with a Hill number of 20

Results

Conformational changes in the dose-response curve

Figure 2 shows the effect of the number of conduction units (n) when there is one conduction pathway ($m = 1$). An increase in the number of conduction units decreases the conformation of the dose-response curve. When n exceeds 100 ($n > 100$), the change becomes negligible. This indicates that the variation in n has little effect on the conduction pathway. The intense cooperativity (Hill number, $[n_H]$ above 20) of clinical anesthesia cannot be achieved by an increase of n values.

Figure 3 shows the effect of the number of conduction pathways (m) when there is one conduction unit in each conduction pathway ($n = 1$). Here again, the conformation of the dose-response curve stayed unchanged when the number exceeded 100 ($m > 100$). A high cooperativity, comparable to in vivo dose-response curves, could not be obtained by an increase in m values either.

Figure 4 shows the dose-response curves when both the number of conduction units and the number of pathways are varied. The conformation of the dose-response curves became steeper with the increase in both parameters. When both numerical values exceeded 10^4, the dose-response curves became comparable to those for in vivo anesthesia.

ED_{95}: the 95% effective concentration

When the Hill number is 20, the 95% effective concentration is calculated to be 1.16-fold of MAC. The MAC_{95} of sevoflurane was also reported to be 1.16 of MAC [7]. Table 1 shows the ratio of EC_{50}^{system} and EC_{95}^{system} for the combination of conduction units and pathways ($EC_{95}^{system}/EC_{50}^{system}$; these values were calculated from Eq. 6). The shadowed area in Table 1 signifies the $EC_{95}^{system}/EC_{50}^{system}$ values that match the in vivo values (1.11–1.21, where we allowed 5% error). The shadowed area shows the number of units above 100 and the number of pathways above 10^6. The results indicate that in vivo anesthesia requires very large numbers of conduction units and pathways.

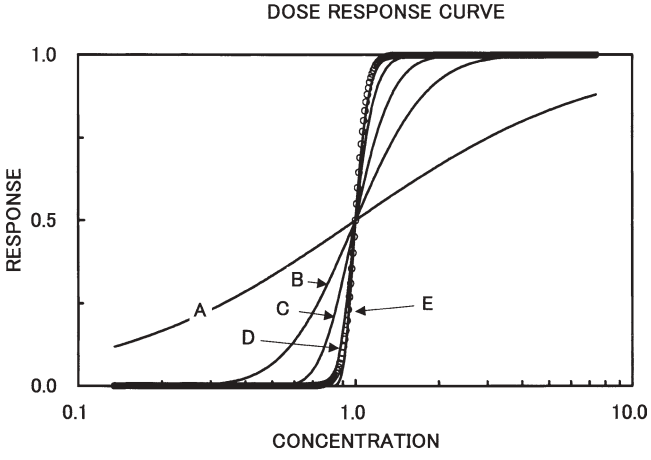

Fig. 4. Effects of the number of conduction pathways (m) and the number of conduction units (n) on dose-response curve in MUMPS model. *Curves A-E* were calculated from Eq. 5, with *A*, $m = 1$ and $n = 1$; *B*, $m = 10^1$ and $n = 10^1$; *C*, $m = 10^2$ and $n = 10^2$; *D*, $m = 10^2$ and $n = 10^4$; and *E*, $m = 10^6$ and $n = 10^6$. *Open circles* represent the dose-response curve calculated from Hill's equation, with a hill number of 20

Table 1. $EC_{95}^{system}/EC_{50}^{system}$ of the combination of conduction units (n) and pathways (m)

		m										
		10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}
n	10^0	1.90×10^1	1.40×10^1	1.36×10^1	1.35×10^1	1.3×10^1	1.35×10^1	1.35×10^1	1.35×10^1	1.35×10^1	1.35×10^1	1.35×10^1
	10^1	4.87	2.24	1.76	1.58	1.48	1.43	1.39	1.37	1.35	1.34	1.33
	10^2	4.37	1.98	1.54	1.38	1.29	1.24	1.20	1.17	1.15	1.14	1.13
	10^3	4.33	1.95	1.52	1.36	1.27	1.22	1.19	1.16	1.14	1.12	1.11
	10^4	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11
	10^5	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11
	10^6	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11
	10^7	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11
	10^8	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11
	10^9	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11
	10^{10}	4.32	1.95	1.52	1.36	1.27	1.22	1.18	1.16	1.14	1.12	1.11

Shadowed area shows $EC_{90}^{system}/EC_{50}^{system}$ values that match the in vivo values (1.11–1.21)

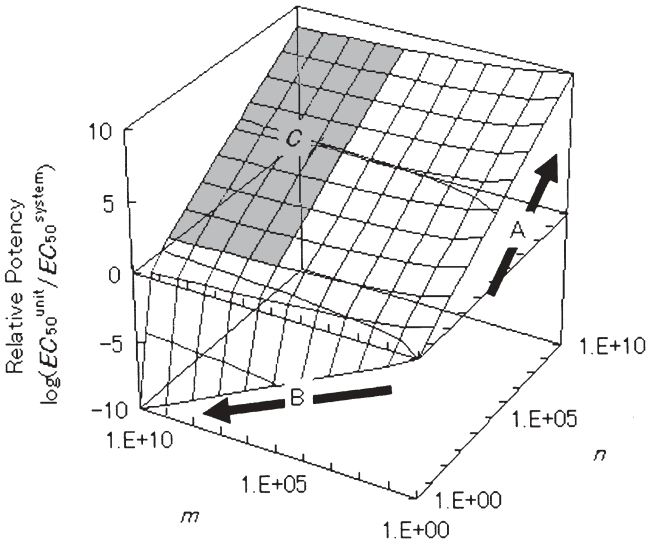

Fig. 5. Effects of the number of conduction pathways (m) and the nubmer of conduction units (n) on $EC_{50}^{unit}/EC_{50}^{system}$ in MUMPS model. $EC_{50}^{unit}/EC_{50}^{system}$ values were calculated from Eq. 5. *Arrow A*, with an increasing number of conduction units (n), the $EC_{50}^{unit}/EC_{50}^{system}$ value increased. *Arrow B*, with an increasing number of conduction paths (m), the $EC_{50}^{unit}/EC_{50}^{system}$ value decreased. The *shaded area* (C) represents $EC_{50}^{unit}/EC_{50}^{system}$, where EC_{95}/EC_{50} was between 1.11 and 1.21

Anesthetic potency on the conduction unit and the total system

Figure 5 shows the ratio between the conduction unit and the whole system ($EC_{50}^{unit}/EC_{50}^{system}$) when their numbers are altered. The vertical axis is plotted in logarithmic scale. In the positive region ($EC_{50}^{unit} > EC_{50}^{system}$) the anesthetic potency on the conduction unit is smaller than the apparent anesthetic potency on the whole system. This means that the action of anesthetics on the system is strong despite the anesthetic effect on each unit being weak.

When the number of conduction units (n) increases, anesthetic action on the whole system becomes more potent. This is because, when one unit fails, each pathway fails to conduct (Fig. 5; arrow A). In contrast, an increase in the number of conduction pathways (m) makes the system less vulnerable to interruption (Fig. 5; arrow B).

Area C in Fig. 5 is calculated from the combination of conduction units and conduction pathways in which the $EC_{95}^{system}/EC_{50}^{system}$ values match the in vivo values (1.11–1.21), and shows that, under these conditions, the log ($EC_{50}^{unit}/EC_{50}^{system}$) is always positive; also the value for $EC_{50}^{unit}/EC_{50}^{system}$ becomes 3.8×10^0 ($m = 10^{10}$ and $n = 10^3$) to 7.1×10^8 ($m = 10^{10}$ and $n = 10^{10}$). Even though the anesthetic effect on each conduction unit is small, the overall effect on the system is large.

Discussion

Eckenhoff and Johansson [8] assumed that mobilization was maintained by multiple elements. They constructed a model composed of multiple elements, where anesthetic effects on each element may be small, but the composite effect may become large. By considering that the actual Hill number of in vivo studies is in the range of 20, they concluded that in vitro EC_{50} values could be larger than the actual MAC. The EC_{50} values of in vitro systems do not necessarily match the EC_{50} values of in vivo systems. With in vitro studies, the EC_{50} values can be higher than MAC. The curves shown in Fig. 1 in the article by Eckenhoff and Johansson [8] were generated by the Hill equation, with changes of the EC_{50} and the Hill number, without a description of the relationship between K_d and EC_{50}. It is a mystery why EC_{50} is 50 μM and the Hill number is 2, when K_d is 1 mM and ten different sites exist with equal additive effects (curve b in the same figure). We notice that the Hill equation is often used for a steep dose response curve, but it is often misused, being used just as a curve-fitting procedure in most cases. The conclusion that there are possible dissociations between MAC and potency at the action sites deserves attention [8], despite the finding that the Hill equation failed to serve an apparent model of anesthesia mechanism [9].

Yamakura et al. [4] and Eger's group [10] assumed that the in vitro actions of anesthetics on each element were a non-quantal (analog) response. It is possible to convert a non-quantal response to quantal by setting a certain borderline and converting it to population distribution (hit or no-hit). Figure 1 in the article by Yamakura et al. [4] shows that the Hill number of the dose-response curves for six oocytes was 1.25. When the data were converted to a categorical response the number increased to 8.4. They stated that the slope of categorical responses for each individual does not depend on the Hill value of the underlying dose-response relationship, but depends on the population distribution. Thus, the steep slope of the MAC response may not necessarily be related to mechanisms of anesthetic actions on targets [4].

Yamakura et al. [4] assumed that the threshold was 0.5, but there is no reason for this assumption. Eger's group [10] discussed the details of the threshold value, and concluded that the threshold should lie within values of 0.1 to 0.9. But this conclusion was not based on necessity, either. They stated that the Hill coefficient of the receptor *probably* exceeded 2, when the threshold was less than 0.1 or greater than 0.9 [10]. There seemed to be no clear reason for this. Furthermore, steep dose-response curve of anesthetics in a non-quantal response have been reported [9].

The observed response of oocytes is a result of the opening and closing of ion channels. The open or closed state of channels is quantal, and the response rates represent the mean value of the probability of an open (or closed) state of every channel on each oocyte. The obtained dose-response curves were already converted from a quantal parameter to a non-quantal parameter. Yamakura et al. [4] and Eger's group [10] converted this non-quantal parameter to quantal one by categorization. This categorization should be done for each oocyte first, using a binomial distribution, and then the probability should be obtained (see Appendix). However, we know neither how much is the threshold value nor how many channels are in each oocyte.

We analyzed the in vivo effects of anesthetics by constructing a model composed of two variables: a conduction unit and a conduction path. We found that the minimum requirements to obtain high cooperativity, which is specific to in vivo anesthesia, were: the number of conduction units had to be above 100 and the number of conduction pathways had to be above 10^6. This indicates that a total of 10^8 units (100×10^6) are required to obtain steep dose-response curves. The appropriateness is considered below.

First, we stress that the unit number (n) does not represent the number of neurons. By considering that anesthesia becomes effective locally when part of the neuron is affected, it can be seen that there are many conduction units in a neuron.

The number of conduction paths (m) in our model represents linear pathways (see Fig. 1). In reality however, neurons ramify into branches and form networks. In these complex systems, it is possible to form many conduction pathways in a single neuron. The assumption of 10^6 conduction paths is not unrealistic. To construct a model with branched neurons means the addition of another variable and only makes the analyses more difficult, without practical benefit for the analyses.

When the strength of the anesthetic effect on the conduction unit (EC_{50}^{unit}) and the whole system (EC_{50}^{system} = MAC) is compared, the contribution of EC_{50}^{unit} is much stronger than that of EC_{50}^{system} under the high cooperativity condition. The numerical value was estimated to be 3.8×10^0 to 7.1×10^8. At clinical anesthetic concentrations, the anesthetic effect on the conduction unit may be very small. The concentrations of aqueous volatile anesthetics in equilibrium with MAC in the gas phase are in the range of 0.19–0.64 mM [11]. Therefore, the EC_{50} for conduction units requires high anesthetic concentrations, of 1 mM or more. The eight-digit difference between the EC_{50}^{unit} and EC_{50}^{system} values was the mathematical value calculated from the model, and may be almost meaningless when we discuss the actual anesthetic potency on the conduction unit. However, these

findings suggested that the anesthetic concentrations for studies on channels, enzymes, membrane proteins, etc. require reconsideration. The effects of these high anesthetic concentrations suggest that the anesthetic effects are directed to the physical properties of the membranes as well as to high-affinity binding sites.

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Appendix

Categorization of the non-quantal response of oocytes

The response rate of the *i*-th oocyte at the anesthetic concentration [*A*], $P_i^o([A])$ is the mean value of the probability of the open (or closed) state of each channel.

$$P_i^o\left([A]\right) = \frac{\sum_{j=1}^{n_i^c} p_{i,j}^c\left([A]\right)}{n_i^c} \tag{A-1}$$

Here, $p_{i,j}^c([A])$ is the probability of the open (or closed) state of the *j*-th channel of the *i*-th oocyte, and n_i^c is the number of channels on the *i*-th oocyte. This equation represents the dose-response curve of each oocyte (for example, Fig. 1 of Yamakura et al. [4] and Fig. 5 of Eger et al. [10]), and the probability of the quantal phenomenon was converted to the non-quantal parameter, the response rate.

Yamakura et al. [4] and Eger's group [10] categorized the non-quantal response of each oocyte by threshold, and the probability of the response of oocytes (or immobility), $P^{IM}([A])$, is

$$P^{IM}\left([A]\right) = \frac{\sum_{i=1}^{N} if\left(\frac{P_i^o\left([A]\right)}{Th} > 1,1,0\right)}{N} \tag{A-2}$$

Here, *Th* is the threshold value of the response, and *N* is the number of oocytes which have been examined. The *if* (*BOOLEAN*, *VALUE 1*, *VALUE 2*) function becomes *VALUE 1*, if the *BOOLEAN* expression is true. If the *BOOLEAN* expression is false, the function becomes *VALUE 2*.

Probability of the quantal response of each oocyte, using binomial distribution

The response rate of each oocyte, $P_i^o([A])$, is

$$P_i^o\left([A]\right) = \sum_{j=n_i^c-n_i^{c,Th}+1}^{n_i^c} \binom{n_i^c}{j} \left\{1 - p_{i,j}^c\left([A]\right)\right\}^{n_i^{c,Th}} \times \left\{p_{i,j}^c\left([A]\right)\right\}^{n_i^c - n_i^{c,Th}} \tag{A-3}$$

Here, $n_i^{c,Th}$ is the number of channels required to classify the response of the oocytes, and is

$$n_i^{c,Th} \cong Th \times n_i^c \tag{A-4}$$

In this case, the response of the oocytes is quantal (on-off, or mobile-immobile). Thus, $P^{IM}([A])$ is the average of $P_i^o([A])$.

$$P^{IM}\left([A]\right) = \frac{\sum_{i=1}^{N} P_i^o\left([A]\right)}{N} \tag{A-5}$$

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