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Examination of voltage gated sodium channel Nav1.4, Nav1.5 and Nav1.6 with regard to different mutations and their sensitivity to associate μo-conotoxin MrVIa

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Contents

1. Introduction

1.1 Course

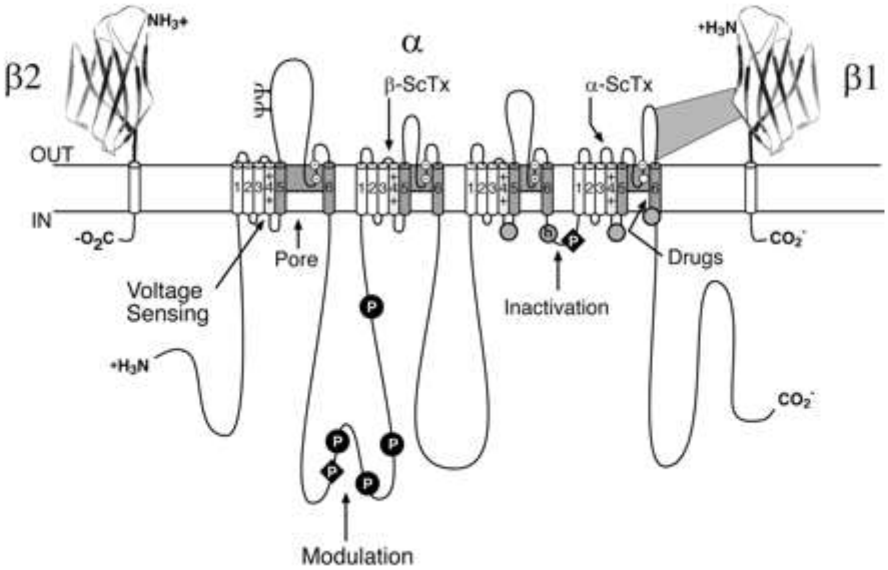
I started my practical work with the voltage gated sodium channel Nav1.4. Than I explored Nav1.6 and at last Nav1.5, too. But at first I practised the different things which I would need. These are on the one side the preparation of oocytes from the cold-blooded south African frog Xenopus laevis. I did this in help of Ms. Rossner. I needed two forceps, a microscope, Bart medium as solution, oocytes of course and a lot of patience. So I learned to remove the transparent skinner from the oocytes after operation and an incubation period in collagenase of 60 minutes. On the other side I practised to inject RNA into the oocytes of the Xenopus leavis also in help of Ms. Rossner. For that I learned to pull and to break glass pipettes and the procedure of injection. During the whole practical and consequently this study I practised these procedures.

For the following work and thus for the data acquirement and analysis I learned to use the two electrodes clamp in help of Prof. S. H. Heinemann, too. So I practised specific kinds of applications in association with the software patch master. Within the last two weeks of my practical I could deal with the software packet fit master where I learned to analyse the kinetic of inactivation. Also in help of Prof. S. H. Heinemann I got an short insight in the mathematics program Igor pro.

Last but not least I used the rest of my free time for intensively literature study.

1.1 Subject

Voltage gated sodium channels are the transmembrane proteins responsible for the initial rising phase of the action potential in electrically excitable cells (Catterall, 2000; Goldin, 2001). Sodium channels consist of a highly processed α subunit, which is approximately 260kDa, associated with auxiliary β subunits (Catterall, 2000). The pore-forming α subunit is sufficient for functional expression, but the kinetics and voltage dependence of channel gating are modified by the β subunits (Catterall et al., 2005) (Fig. 1).



(Fig. 1; Catterall et al.)

In mammals, there are nine sodium channel α-subunit isoforms named Nav1.1 - Nav1.9 (Goldin 2002; Goldin et al., 2000). One of these, Nav1.4 is expressed at high levels in adult skeletal muscle and low levels in neonatal skeletal muscle. The channel type Nav1.5 is located predominantly at cardiac myocytes and Nav1.6 channel is one of the most abundant voltage gated sodium channels in the brain (Catterall et al., 2005). But the β effect is only demonstrable at Nav1.4 and Nav1.6. Not at Nav1.5.
Nav1.4, Nav1.5 and Nav1.6 are interest in for my practical work.

1.2 Practical work

For each of these channels existed four different RNA's which express differ units but always with an α subunit in the cell surface membrane. These are at first only an α unit as control, second an α unit and a transmembrane unit, third an α unit and an auxiliary β1 subunit and fourth also an α unit and an auxiliary β1 unit but with a longer linker, called L-β1. The last tree units are associated with a toxin from predatory cone snails (μo-conotoxin; MrVIa).
The cone snails or cone shells (*Conidae*) are marine snails found in coral reefs. Cone snails can grow up to 23 cm in length and are found in tropical waters (Fig. 2). There are about 500 different species. They are carnivorous, generally eating marine worms, small fish, and even other molluscs. Because cone snails are slow-moving, they use a venomous harpoon (actually a toxoglossan radula) to catch fast-moving prey such as fish. The venom of some species is powerful enough to kill a human being. (Wikipedia, 2006)

Geography cone, *Conus geographus*

(Fig. 2)

After expression the toxin was outside of the cell surface membrane.

The cell surface membrane is the cell's outer limiting membrane. It is very thin (varying from 7-10nm in thickness; the membrane is 7-10nm wide) and composed chiefly of a fluid bilayer of phospholopide molecules with protein molecules dispersed in it. The proteins float in the bilayer, forming a constantly changing mosaic pattern (E. Klein, 2005).

In this study, I compared the properties of Nav1.4, Nav1.5, Nav1.6 and their mutations with regard to the efficiency of μo-conotoxin (MrVIa). I also explored whether the influence of μo-conotoxin is depend on his location.

2 Materials and Methods

2.1 Expression of the sodium channels in the south African frog Xenopus laevis oocytes

I used for Expression of the sodium channels Nav1.4, Nav1.5 and Nav1.6 the south African frog Xenopus laevis oocytes. For that I carried out four lines. Two for Nav1.4 and one for each of the other two. I injected for each channel four different RNA's (Tab. 1). I measured every line twice, only the third line once. Thus I carried out seven measurements during my practical.

	O.p. NR.	Injection	Measurement	RNA	Relation
1st line Nav1.4	1082	19.04.2006	20.04.2006 21.04.2006	Nav1.4:H2O:H2O Nav1.4:pCS2-MrVIA-TM-EGFP:H2O Nav1.4:pCS2-MrVIA-ß1-EGFP:H2O	1:02:02 1:02:02 1:02:02
2nd line Nav1.4	1083	24.04.2006	25.04.2006 26.04.2006	Nav1.4:H2O:H2O Nav1.4:pCS2-MrVIA-TM-EGFP:H2O Nav1.4:pCS2-MrVIA-ß1-EGFP:H2O Nav1.4:pCS2-MrVIA-L-ß1-EGFP:H2O	1:02:02 1:02:02 1:02:02 1:02:02
3rd line Nav1.6	1085	03.05.2006	04.05.2006	Nav1.6:H2O:H2O Nav1.6:pCS2-MrVIA-TM-EGFP:H2O Nav1.6:pCS2-MrVIA-ß1-EGFP:H2O Nav1.6:pCS2-MrVIA-L-ß1-EGFP:H2O	1:02:02 1:02:02 1:02:02 1:02:02
4th line Nav1.5	1086	09.05.2006	10.05.2006 11.05.2006	Nav1.5:H2O:H2O Nav1.5:pCS2-MrVIA-TM-EGFP:H2O Nav1.5:pCS2-MrVIA-ß1-EGFP:H2O Nav1.5:pCS2-MrVIA-L-ß1-EGFP:H2O	1:02:02 1:02:02 1:02:02 1:02:02

(Tab. 1)

2.2 Electro physiological recording

I used for Electro physiological recording the two-electrode voltage clamp. The recording solution was frog's ringer without supplements. The electrodes were coated by electrolyse in a KCl solution. I used filament glasses to pull the glass pipettes.

2.3 Data analysis

Data analysis was performed using the patch master and fit master software packet. Peak conductance was calculated by using the patch master after a period of 60 sec. The kinetics of inactivation were analysed by fitting the current traces between short after the peak current and the end of the depolarisation with an exponential equation. I calculated the several results (main value, standard derivation, root and t-test) in Microsoft Excel and created the graphs there, too.

3 Results

I started with a membrane potential of -100 mV. Then I used a pulse generator of -60mV – 30mV in 10 steps of 10 mV for Data acquirement after a period of 60 sec. I was interest in the peak current of each measurement and the kinetics of –10mV or –40mV (Tab.2 – Tab.5).

Nav1.4			**Current in nA**			**Kinetic in ms**	**(-10mV)**	
			Ctrl	TM	β1	Ctrl	TM	β1
1st line	1st Measurement	Mean value	-817.9	-61.7	-306.4	14.1	17	5.9
		Standard deviation	1066.3	37.3	168.2	1.7	3.6	2.4
		Number	17	17	16	17	15	16
		Root	258,6	9.0	42.0	0.4	0.9	0.6
	2nd Measurement	Mean value	-391.65	-48.0	-484.56	13.05	11.62	3.77
		Standard deviation	434.43	30.82	759.92	2.09	1.72	1.17
		Number	21	20	23	21	21	22
		Root	94.8	6.9	158.5	0.5	0.4	0.2

(Tab. 2)

Nav1.4			**Current in nA**				**Kinetic in ms**	**(-10mV)**		
			Ctrl	TM	β1	L-β1	Ctrl	TM	β1	L-β1
2nd line	1st Measurement	Mean value	-1346.0	-101.6	-1953.9	-351.5	10.3	10.4	4.1	2.9
		Standard deviation	1655.1	64.3	1053.4	470.5	1.2	1.9	1.5	1
		Number	14	14	14	14	14	12	12	11
		Root	442.3	17.2	281.5	125.7	0.3	0.6	0.4	0.3
	2nd Measurement	Mean value	-5701.9	-210.6	-7874.3	-2432.0	11.2	9.9	3.7	1.3
		Standard deviation	5514.0	136.2	4146.9	1403.0	1.5	2.3	1	0.3
		Number	11	13	14	11	11	12	14	11
		Root	1662.5	37.8	1108.3	423.0	0.5	0.7	0.3	0.1

(Tab. 3)

Nav1.6			**Current in nA**				**Kinetic in ms**	**(-10mV)**		
			Ctrl	TM	β1	L-β1	Ctrl	TM	β1	L-β1
3rd line	1st Measurement	Mean value	-1863.4	-247.0	-1606.3	-878.7	6.4	8.8	1	1.1
		Standard deviation	1862.3	156.1	1394.4	1060.7	1.6	2.6	0.3	0.5
		Number	24	21	27	21	24	20	26	18
		Root	380.1	34.1	268.4	231.5	0.3	0.6	0.1	0.1

(Tab. 4)

Nav1.5			Current in nA				Kinetic in ms		(-40mV)	
			Ctrl	TM	β1	L-β1	Ctrl	TM	β1	L-β1
4th line	1st Measurement	Mean value	-1185.1	-1819.6	-2151.0	-386.6	4.4	4.1	3.3	7.4
		Standard deviation	1012.5	1402.4	2411.4	197.8	2.4	3.0	2.3	3.8
		Number	13	14	14	13	14	14	14	13
		Root	280.8	374.8	644.5	54.9	0.6	0.8	0.6	1.0
	2nd Measurement	Mean value	-3308.3	-9522.7	-18244.5	-2734.6	2.7	1.4	1.4	4.3
		Standard deviation	1695.2	4260.8	11686.3	1926.1	1.3	0.6	0.6	2.0
		Number	12	11	11	17	13	11	11	17
		Root	489.4	1284.7	3523.6	467.1	0.4	0.2	0.2	0.5

(Tab. 5)

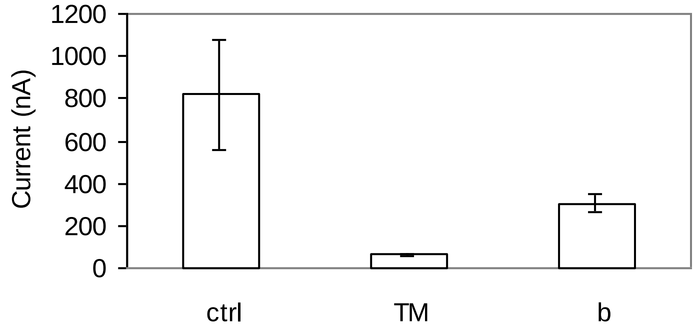
4 Discussion

4.1 Nav1.4 channel

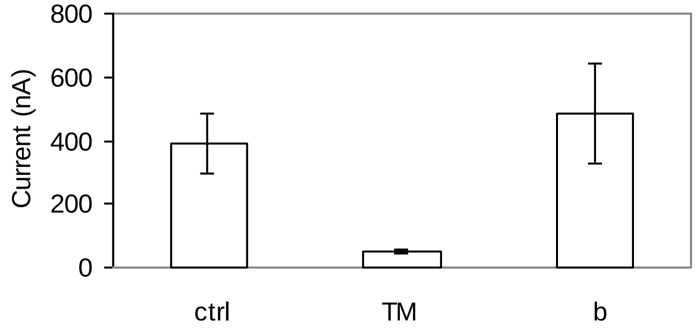
4.1.1 Current

Significant of Nav1.4 channel is the following. With regard to their relations both the first and the second line are similar (Tab. 2 / Tab. 3). The current results show solid values. The TM unit seem to block the channel (mean values = -61.7nA; -48.0nA; -101.6nA; -210.6nA). These results raise the possibility that TM influence the current flow.
However, the ctrl group did it not and the β1 cells very probably, too. Because the current flow of β1 (mean values = -306.4nA; -484.56nA; -1953.9nA; -7874.3nA) cells are the same and mostly higher than the current flow of ctrl cells. The reason why these results differ between the first and the second line could be in the different injection or expression of RNA. Specially the expression of RNA could be the reason why the results differ in these lines.

1st line / 1st Measurement



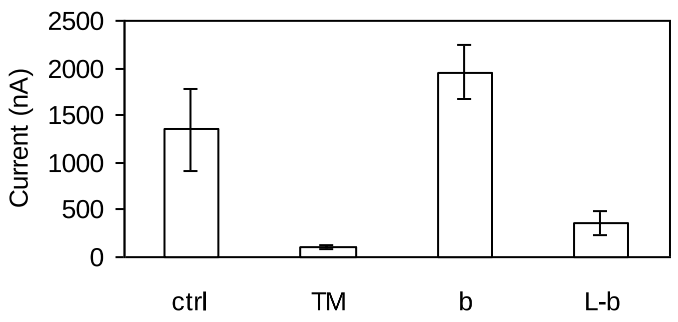
1st line / 2nd Measurement



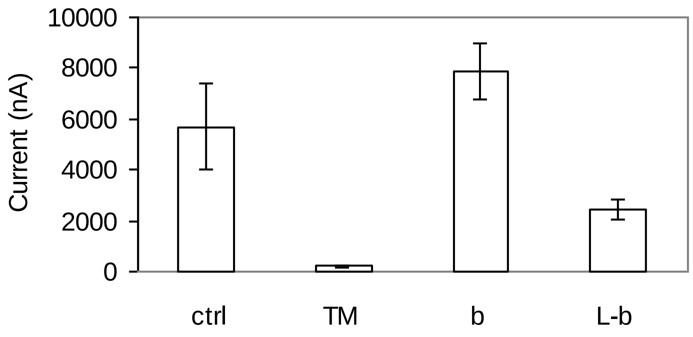
With regard to see what happen when β1 is associate with a longer linker I checked it in a second line. Although all values are bigger here than the values in the first line the relations are the same. The oocytes with L-β1 showed less values (mean values = -351.5nA; -2432.0nA). These results are less than the results of β1 cells, but higher than the results of TM cells. These results raise the possibility that L-β1 influence the current flow probably, too. What could happen? It is possible that the toxin on the short β1 linker could not reach the channel but the toxin on the Lβ-1 did it.

In the view of the peak current it is not clear why there are differences between the ctrl and the β1 group relations in the measurements of the lines. However, in the second line it is clear visible that the cells which prepared with β1 RNA showed more peak current than they without auxiliary unit. Probably is the influence of β1 in the second line more clearly.

2nd line / 1st Measurement



2nd line / 2nd Measurement

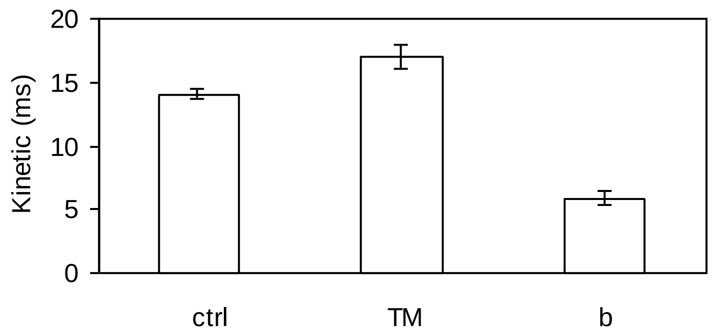


4.1.2 Kinetic

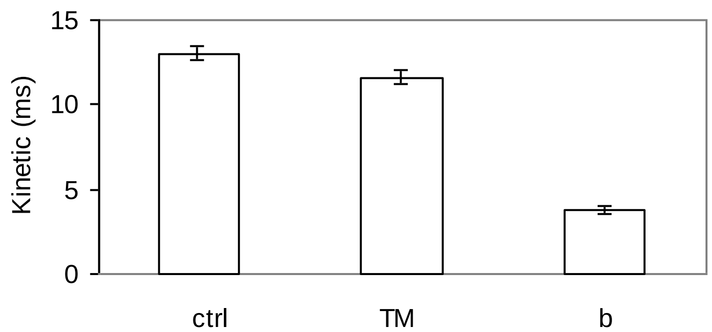
However, the kinetic values show an other relation. Here I did the following finding. The results of the TM (mean values = 17.0ms; 11.62ms; 10.4ms; 9.9ms) and the ctrl cells (mean values = 14.1ms; 13.05ms; 10.3ms; 11.2ms) are apparently similar. Both allowed the conclusion that they needed more time for channel inactivation than the β1 cells.

Because in contrast with these results the β1 cells (mean values = 5.9ms; 3.77ms; 4.1ms; 3.7ms) were more than the half faster for moving toward to the resting potential. These results raise the possibility that β1 influence the kinetic. This stands in accordance with previous works.

1st line / 1st Measurement

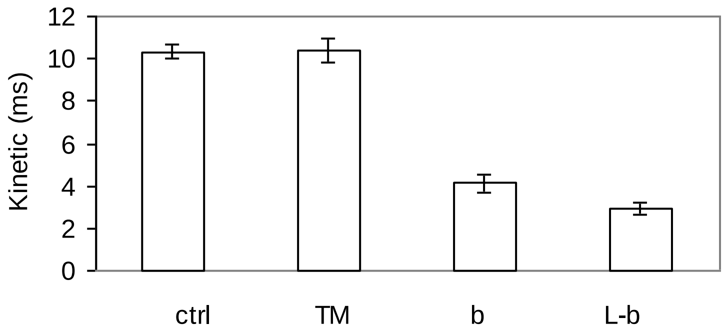


1st line / 2nd Measurement

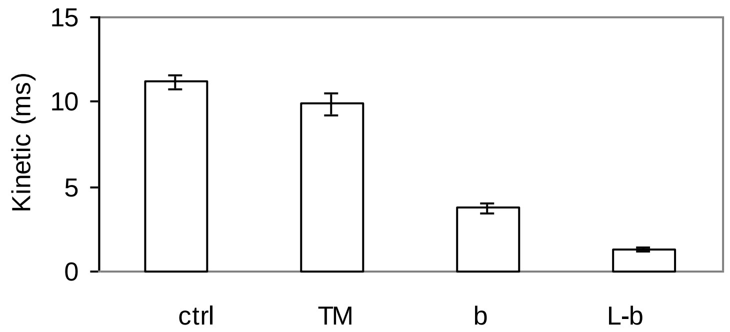


Within the second line I calculated the kinetic of L-β1, too. The results (main value = 2,9ms; 1,3ms) were less than the results of β1 cells. These results raise the possibility that L-β1 influence the kinetic probably, too.

2nd line / 1st Measurement



2nd line /2nd Measurement



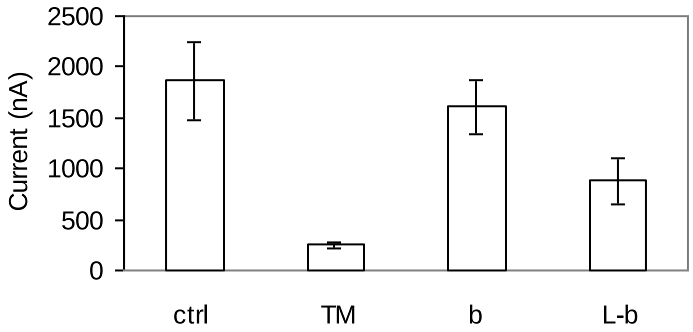
4.2 Nav1.6 channel

4.2.1 Current

With regard to the peak current the results show a clear image (Tab. 4). The transmembrane unit associate with the toxin MrVIa injected into oocytes seemed to inhibit the depolarisation nearly complete (mean value = 247.0nA). The localisation could be the reason why.
Also the oocytes with L-β1 were less excitable (main value = 878.7nA) but more than TM.

Only the ctrl group (main value = 1863.4nA) and the β1 (mean value = 1606.3nA) cells showed clearly current flows. Therefore, it is possible to say that the toxin had less, maybe no influence in combination with β1.

3rd line / 1st Measurement

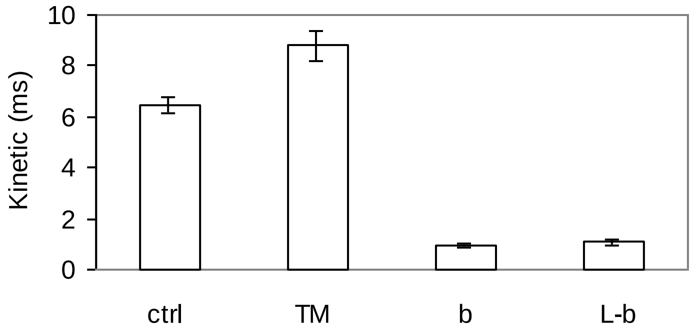


4.2.2 Kinetic

Also the effects with regard to the kinetics of the different mutations and their ctrl group of the Nav1.6 channel give information on their properties. The auxiliary subunits β1 (mean value = 1.0ms) and L-β1 (mean value = 1.1ms) seemed to support the channel inactivation. These results raise the possibility that β1 and L-β1 influence the kinetic toward to the resting potential.

The acquired results speak a clear language and stand in contrast to the values of the ctrl (main value = 6.4ms) and TM group (main value = 8.8ms). These two needed clear more time for inactivation toward to the resting potential. However, TM cells needed more time than ctrl cells.

3rd line / 1st Measurement

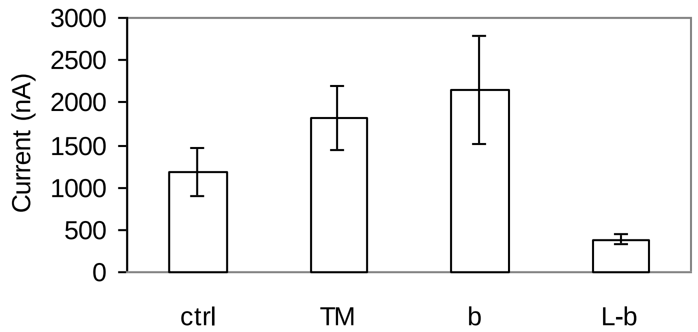


4.3 Nav1.5 channel

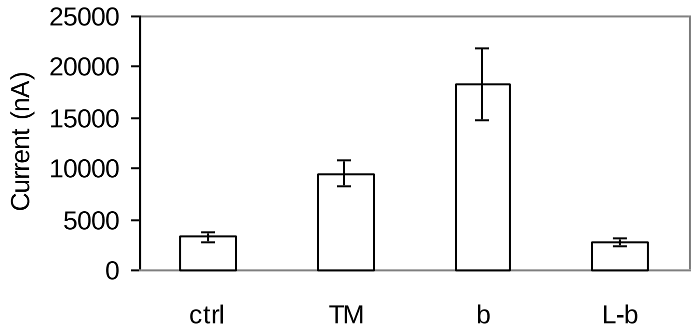
4.3.1 Current

It may be that the values of peak current show that oocytes with a L-β1 unit (mean value = -386.6nA; -2734.6nA) blocked the channel pore (Tab. 5). The long linker could be the reason why. In contrast at this result are the values of oocytes with an α unit as ctrl group (mean value = -1185.1nA; -3308.3nA), a TM (mean value = -1819.6; -9522.7nA) or a β1 (mean value = -2151.0nA; -18244.5nA) unit. These tree injected RNA's expressed channel types which didn't block by associated toxin. May be the toxin couldn't block these channels.
Within the second measuring the results are similar to they of the first measuring but clear higher. Especially β1 shows it. It is possible that the expression is a progressive process and thus after two days higher. That way could explain the influence of time. The strong change of L-β1 is striking. It is possible that a part of the toxin took off. But the result raises the possibility that L-β1 influence the current flow within the second measurement, too.

4th line / 1st Measurement



4th line / 2nd Measurement

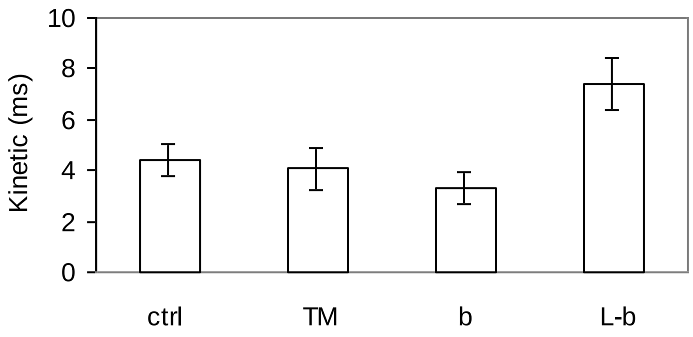


4.3.2 Kinetic

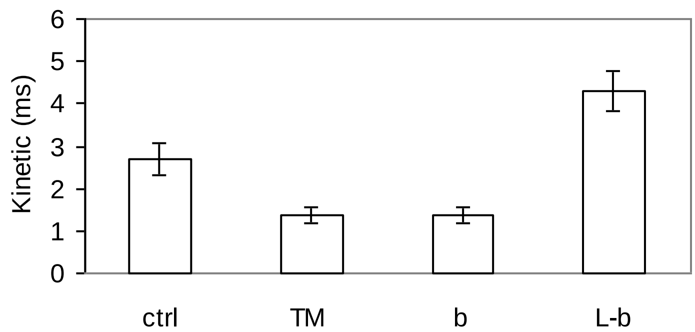
The ctrl (mean value = 4.4ms; 2.7ms) and TM (mean value = 4.1ms; 1.4ms) results are similar during the first measurement. The β1 value (mean value = 3.3ms; 1.4ms) is less than these two cell groups and the L-β1 (mean value = 7.4ms; 4.3ms) higher.

During the second measurement the values are a little bit differ. But the relation is the same. These results raise the possibility that β1 influence the kinetic less. Certainly the numbers of ctrl are higher than they of the TM and β1 but not considerable. On the whole I could only show that the numbers of L-β1 higher than the they of the others. L-β1 need thus clear more time for moving toward to the resting potential.

4th line / 1st Measurement



4th line / 2nd Measurement



5 Summery

This practical work was very interesting and informatively for me. The comparison of these tree voltage gated sodium channels showed that. Whereas the channel type Nav1.4 and the channel type Nav1.6 showed similar behaviour the channel type Nav1.5 showed other properties with regard to the current flow of TM cells. The conclusion could be that the μo-conotoxin within of the TM unit of voltage gated sodium channel Nav1.4 and Nav1.6 block the channel. The results raise the possibility that TM influence the current flow of these two channel types but not the channel Nav1.5.

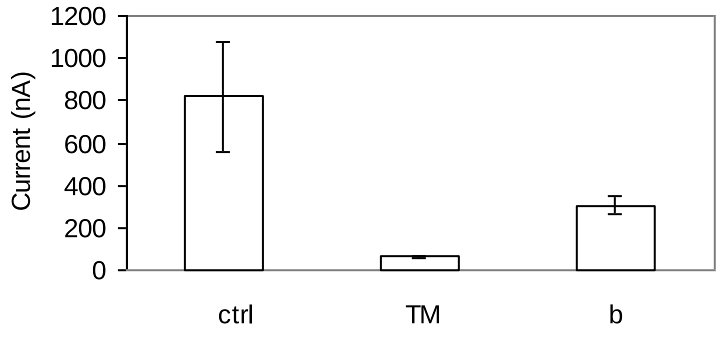
The second interesting fact is that L-β1 units influence the current flow probably, too. But in contrast to TM cells the results showed an effect also during measurement with Nav1.5. The results raise the possibility that L-β1 influence the current flow of all tree channel types but not so strong like TM. Because the mean values of L-β1 cells were always higher than the values of TM cells but less than the value of ctrl and β1 cells within the second and the third line.

The third interesting result is a fact who shows also a difference between Nav1.4 and Nav1.6 on one side and Nav1.5 on the other side. Within the first, the second and the third line it is clear visible that β1 cells and L-β1 cells needed clear less time for channel inactivation than ctrl or TM cells. However, within the fourth line β1 cells needed less time for inactivation, too. But L-β1 cells needed clear more time than the other cells for moving toward to the resting potential. The β1 unit seemed to support the channel inactivation at the channels Nav1.4, Nav1.6 and Nav1.5 as an auxiliary subunit also with the associated toxin. But the same unit with a longer linker did not support the inactivation of the voltage gated sodium channel Nav1.5.

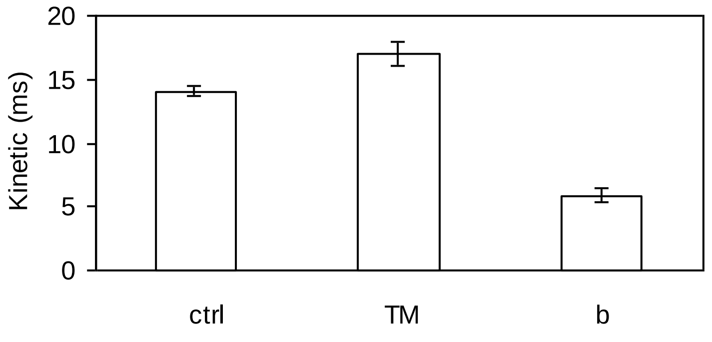
To finish this work, it is possible to say that the conotoxin MrVIa influence the current flow. But the effect is depending from the kind of RNA and thus the kind of localisation.

6 Appendix: Tables, Results, Graphs

Nav 1.4	**ctrl**	**TM**	**b1**
1st line /	656	39,7	272
1st Measuring	3780	117	372
	1060	30,8	210
	1060	49	128
	507	64,5	141
	322	16,7	
	656	44,4	188
	340	41	342
Current in nA	320	53,7	357
	431	40,2	259
	354	40,7	277
	220	61	410
	256	67,7	296
	245	75,9	850
	264	46,6	160
	3320	85,5	296
	114	175	345
Mean value	817,9	61,7	306,4
Standard derivation	1066,3	37,3	168,2
Number	17	17	16
Root	258,6	9,0	42,0
t-test			
ctrl vs TM/b1		0,010	0,068
TM vs b1			0,000

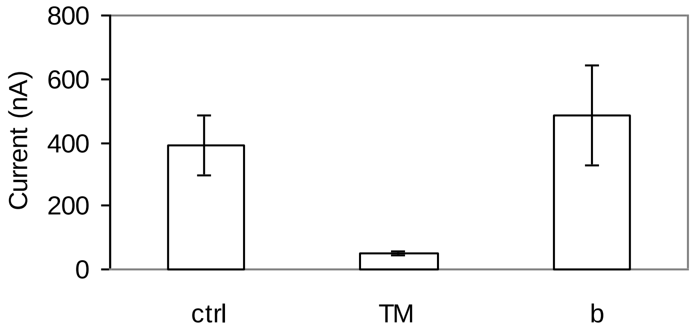


Nav 1.4	ctrl	TM	b1
1st line /	16,28	17,79	7,709
1st Measuring	11,75	18,95	7,722
	15,29	14,82	11
	12,27	20,52	6,202
	16,31	25,95	5,918
	16,87		
	13,56	13,78	9,775
	14,62	18,3	5,45
Kinetic in ms	15,21	17,79	4,114
at -10mV	13,98		4,463
	14,02	16,73	6,644
	14,56	10,35	2,905
	13,77	18,14	6,667
	14,17	13,49	3,395
	13,8	14,1	4,212
	10,86	18,02	5,202
	12,06	15,61	2,73
Mean value	14,1	17,0	5,9
Standard derivation	1,7	3,6	2,4
Number	17	15	16
Root	0,4	0,9	0,6
t-test			
ctrl vs TM/b1		0,011	0,000
TM vs b1			0,000

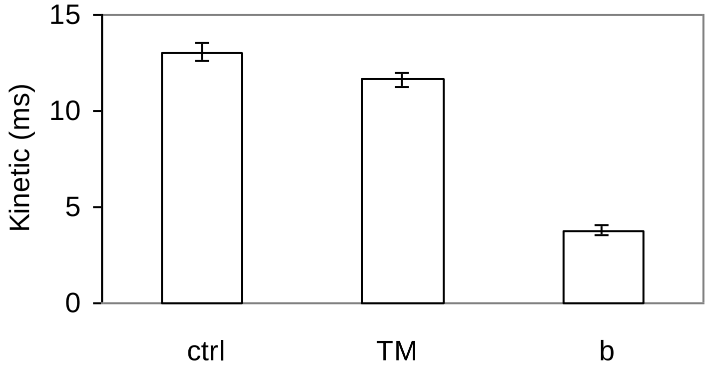


Nav 1.4	**ctrl**	**TM**	**b1**
1st line /	825	22,2	223
2nd Measuring	101	13,7	506
	135	34,9	92,1
	99,6	80,3	259
	361	26,1	210
	288	56,6	388
	87,4	81,8	266
	23,3	13	202
Current in nA	415		6,682
	94,4		
	184	39,4	97,2
	130	26,6	196
	1070	58,4	300
	656	98,9	147
	234	26,1	199
	185	15,3	415
	620	40,9	344
	509	57,2	157
		24,1	1420
	123	46,8	430
	1860	70,6	198
	224	127	585
			3700
			804

Mean value	391,65	48,00	484,56
Standard derivation	434,95	30,96	760,40
Number	21	20	23
Root	94,9	6,9	158,6
t-test			
ctrl vs TM/b1		0,002	0,618
TM vs b1			0,012



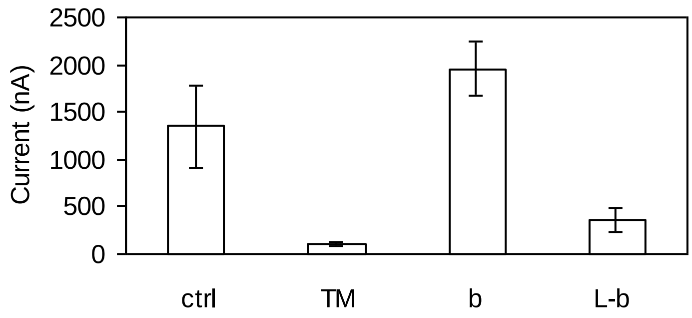
Nav 1.4	**ctrl**	**TM**	**b1**
1st line /	16,82	14,8	5,195
2nd Measuring	14,25	12,68	2,282
	14,96	12,29	6,093
	14,73	12,32	5,583
	13,24	13,19	3,333
	11,34	11,21	2,698
	16,33	10,94	3,326
	17,73	10,48	4,84
Kinetic in ms	12,63	13,63	
at -10mV	11,64		
	12,26	14	4,025
	11,91	10,15	3,426
	13,67	11,92	2,747
	11,37	12,14	3,587
	11,29	9,963	2,702
	12,45	9,589	2,977
	10,51	11,65	4,109
	11,5	9,154	5,105
		11,35	1,816
	9,953	11,39	5,366
	12,47	13,3	3,047
	12,92	7,819	4,231
			3,57
			2,808
Mean value	13,05	11,62	3,77
Standard derivation	2,09	1,72	1,17
Number	21	21	22
Root	0,5	0,4	0,2
t-test			
ctrl vs TM/b1		0,020	0,000
TM vs b1			0,000



Nav 1.4	**ctrl**	**TM**	**b1**	**L-b1**
2nd line /	740	65,2	2070	231
1st Measuring	1170	69,1	2180	14,9
	275	44,8	552	170
	288	98,7	1500	19,7
	759	53	1340	183
	1590	106	1690	207
	319	82	2550	422
Current in nA	2810	88	1780	273
	1450	177	1380	271
	572	122	783	430
	1150	125	1800	240
	244	268	1600	180
	6570	123	3520	1930
	907	0	4610	350

Mean value	1346,0	101,6	1953,9	351,5
Standard derivation	1655,1	64,3	1053,4	470,5
Number	14	14	14	14
Root	442,3	17,2	281,5	125,7

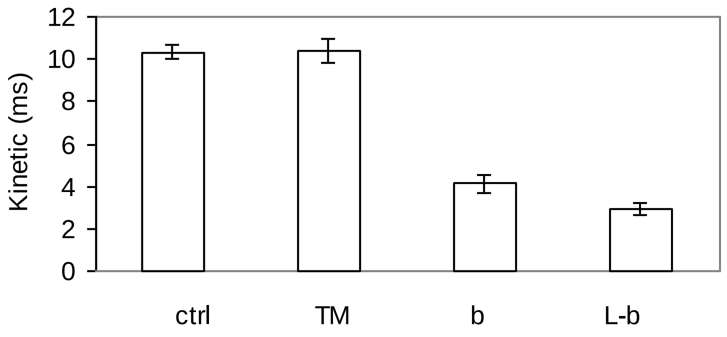
t-test				
ctrl vs TM/b1/L-b1		0,015	0,259	0,047
TM vs b1/L-b1			0,000	0,070
b1 vs L-b1				0,000



Nav 1.4	ctrl	TM	b1	L-b1
2nd line /	10,12	7,347		2,302
1st Measuring	10,72	10,69	4,99	
	12,07		3,424	4,436
	10,6	13,31	6,869	
	10,91	13,74	4,409	2,414
	9,599	7,925	4,896	3,513
	11,34	11,39	5,681	4,54
Kinetic in ms	7,987	9,778	4,481	3,132
at -10mV	9,138	11,06	3,818	2,348
	11,21	9,117	4,176	2,85
	8,984	9,117	3,055	2,773
	12,35	10,33	2,739	2,493
	10,1	11,23	1,233	1,322
	9,223			

Mean value	10,3	10,4	4,1	2,9
Standard derivation	1,2	1,9	1,5	1,0
Number	14	12	12	11
Root	0,3	0,6	0,4	0,3

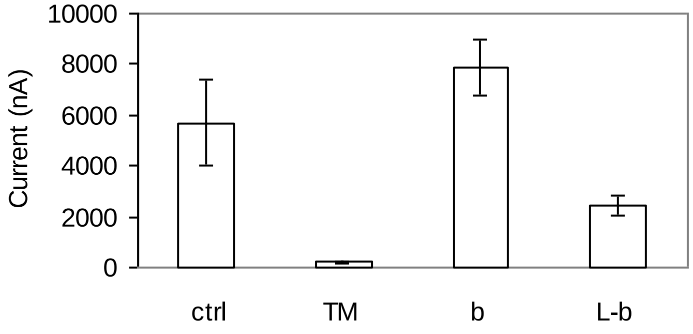
t-test				
ctrl vs TM/b1/L-b1		0,869	0,000	0,000
TM vs b1/L-b1			0,000	0,000
b1 vs L-b1				0,026



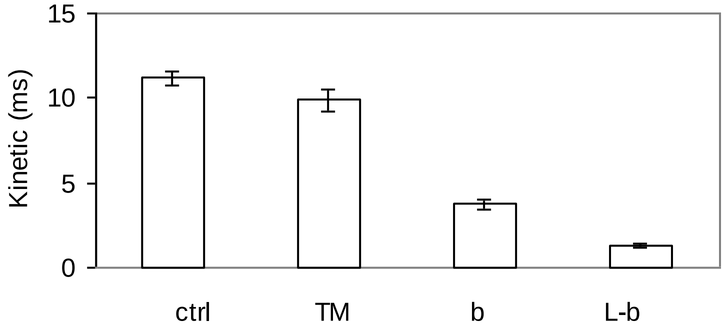
Nav 1.4	**ctrl**	**TM**	**b1**	**L-b1**
2nd line /	1401	288	3920	892
2nd Measuring	1620	56,1	13800	2080
	4360	35,3	12600	5080
	7730	188	5900	1530
	2310	154	4040	2250
	2520	113	10100	1180
	4530	416	8590	3200
Current in nA	4940	107	7360	1380
	1310	426	6510	4830
	18000	208	2340	1870
	14000	183	13800	2460
		143	1750	
		421	12400	
			7130	

Mean value	5701,9	210,6	7874,3	2432,0
Standard derivation	5514,0	136,2	4146,9	1403,0
Number	11	13	14	11
Root	1662,5	37,8	1108,3	423,0

t-test				
ctrl vs TM/b1/L-b1		0,008	0,291	0,082
TM vs b1/L-b1			0,000	0,000
b1 vs L-b1				0,000

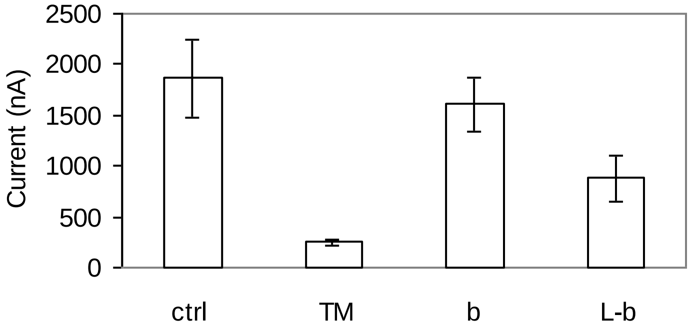


Nav 1.4	**ctrl**	**TM**	**b1**	**L-b1**
2nd line /	15,49	14,6	4,686	1,91
2nd Measuring	11,15	11,45	4,998	1,195
	11,4		4,757	0,949
	10,97	8,313	4,535	1,547
	10,23	8,412	2,901	1,438
	11,12	13,78	4,779	0,991
	10,31	8,811	3,578	1,154
Kinetic in ms	10,71	10,68	3,397	1,459
at -10mV	10,78	8,434	4,225	1,296
	10,71	7,515	2,274	1,294
	9,977	9,095	3,506	1,137
		9,392	2,757	
		8,438	1,893	
			4,066	
Mean value	11,2	9,9	3,7	1,3
Standard derivation	1,5	2,3	1,0	0,3
Number	11	12	14	11
Root	0,5	0,7	0,3	0,1
t-test				
ctrl vs TM/b1/L-b1		0,131	0,000	0,000
TM vs b1/L-b1			0,000	0,000
b1 vs L-b1				0,000

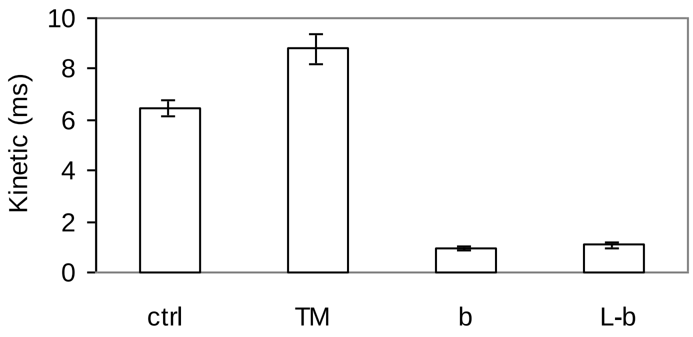


Nav 1.6	**ctrl**	**TM**	**b1**	**L-b1**
3rd line /	444		171	367
1st Measuring	743	110	2090	4580
	464	188	298	804
	1270	183	719	108
	724	123	494	5,5
	1340	97,3	570	608
	275	46,8	1760	200
	324	71,1	404	589
	400	228	1390	264
	925	240	950	370
	1980	124	526	110
	861	220	1460	358
Current in nA	1020	309	6400	622
	3300	415	788	894
	1480	517	761	1080
	994	104	1420	862
	5550	239	1240	707
				926
	2380	450	1560	538
	7620	348	1700	2990
	817	341	1320	1470
	3270		1020	
	2870	201	2290	
	4710	632	3710	
	961		1360	
			1230	
			3860	
			3880	

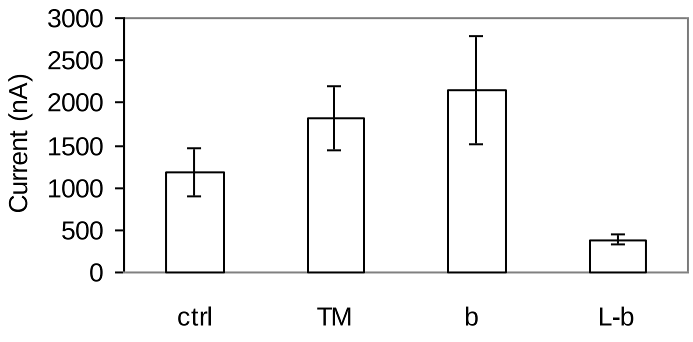
Mean value	1863,4	247,0	1606,3	878,7
Standard deviation	1862,3	156,1	1394,4	1060,7
Number	24	21	27	21
Root	380,1	34,1	268,4	231,5
t-test				
ctrl vs TM/b1/L-b1		0,000	0,584	0,033
TM vs b1/L- b1			0,000	0,013
b1 vs L-b1				0,046



Nav 1.6	ctrl	TM	b1	L-b1
3rd line /	10,35		1,952	1,411
1st Measuring	8,18	11,28	0,769	0,872
	8,469	13,34		0,812
	7,584	10,17	0,806	
	7,55	7,534	1,033	
	6,503	10,36	0,963	1,181
	6,79	13,58	0,869	3,011
	8,095	14,41	1,639	1,145
	6,941	8,738	0,9	0,877
	6,779	7,575	0,972	1,328
	6,94	7,801	1,449	1,043
	6,901	7,834	0,937	1,143
Kinetic in ms	6,674	7,882	0,497	0,766
at -10mV	5,399	9,105	0,961	0,979
	6,133	7,196	0,691	0,994
	6,257		0,705	0,759
	5,754	8,389	0,959	1,047
	5,296	7,617	1,122	0,765
	3,326	6,03	1,255	0,63
	6,575	6,374	0,69	0,886
	4,513		0,639	
	4,972	5,459	0,629	
	3,365	5,51	0,873	
	5,06		0,801	
			0,955	
			0,758	
			1,288	
Main value	6,43	8,81	0,97	1,09
Standard deviation	1,60	2,63	0,33	0,52
Number	24	20	26	18
Root	0,33	0,59	0,06	0,12
t-test				
ctrl vs TM/b1/L-b1		0,001	0,000	0,000
TM vs b1/L-b1			0,000	0,000
b1 vs L-b1				0,373



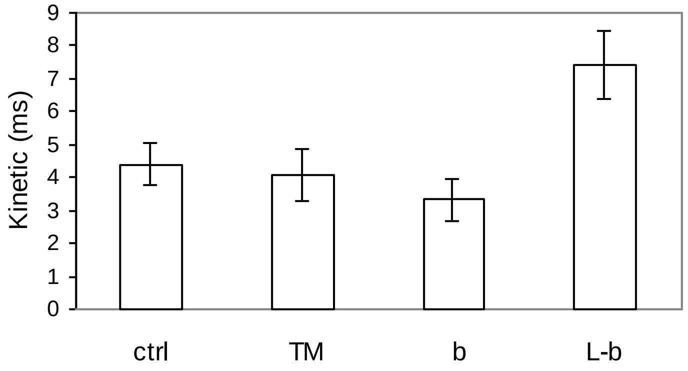
	ctrl	TM	b1	L-b1
Nav 1.5	**ctrl**	**TM**	**b1**	**L-b1**
4th line /		167	337	120
1st Measurement	433	2740	2430	158
	422	2640	31,7	345
	1760	1690	1340	196
	329	493	289	560
	2230	1690	1230	307
	91,3	647	707	289
	716	717	2560	419
Current in nA	1220	1560	2630	386
	756	1280	9370	694
	3740	5170	4560	247
	479	3900	859	598
	1630	1960	1360	
	1600	820	2410	707
Mean value	1185,1	1819,6	2151,0	386,6
Standard derivation	1012,5	1402,4	2411,4	197,8
Number	13	14	14	13
Root	280,8	374,8	644,5	54,9
t-test				
ctrl vs TM/b1/L-b1		0,188	0,187	0,015
TM vs b1/L-b1			0,661	0,002
b1 vs L-b1				0,017



Nav 1.5	**ctrl**	**TM**	**b1**	**L-b1**
4th line /	8,215	11,71	9,569	11,02
1st Measurement	6,151	3,812	2,396	16,63
	8,893	6,841		7,817
	3,774	4,322	4,002	5,665
	7,418	8,057	6,594	5,166
	4,11	1,625	2,733	8,22
	4,086	2,591	3,723	11,35
	3,842	5,123	3,399	8,517
Kinetic in ms	1,755	1,922	1,845	3,743
at -40mV	2,232	3,051	1,095	4,903
	1,609	1,525	1,421	4,763
	3,914	1,145	2,835	3,343
	3,43	2,326	3,43	
			1,063	
	2,031	2,77	2,345	5,212

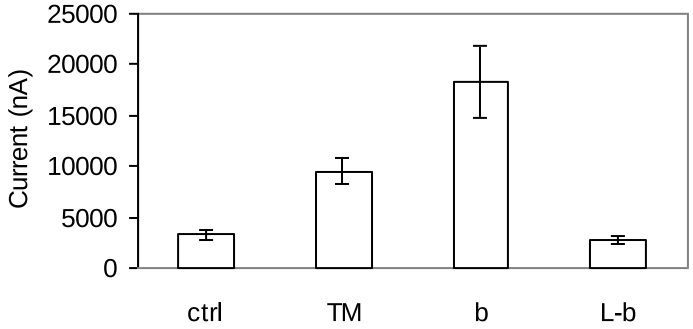
Mean value	4,4	4,1	3,3	7,4
Standard derivation	2,4	3,0	2,3	3,8
Number	14	14	14	13
Root	0,6	0,8	0,6	1,0

t-test			
ctrl vs TM/b1/L-b1	0,749	0,237	0,023
TM vs b1/L-b1		0,470	0,018
b1 vs L-b1			0,003



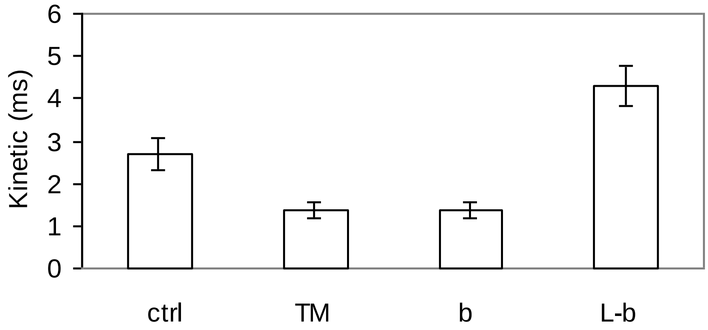
Nav 1.5	**ctrl**	**TM**	**b1**	**L-b1**
4th line /	1980			945
2nd Measurement				717
				927
				2120
				2380
	2460	10100	11000	1460
				2370
	2620	6700	19400	1880
	2480	15200	11300	8620
Current in nA	3100	13300	14100	5690
	3160	3670	11300	3410
	2260	15600	38800	1630
	2630	9130	30400	2980
	4920	8830	34700	2310
	2310	3480	19200	3160
	3720	6340	2220	2580
	8060	12400	8270	3310
Mean value	3308,3	9522,7	18244,5	2734,6
Standard derivation	1695,2	4260,8	11686,3	1926,1
Number	12	11	11	17
Root	489,4	1284,7	3523,6	467,1
t-test				
ctrl vs TM/b1/L-b1		0,001	0,002	0,404
TM vs b1/L-b1			0,037	0,000

b1 vs L-b1	0,001



Nav 1.5 4th line / 2nd Measurement	**ctrl**	**TM**	**b1**	**L-b1**
	5,558			8,625
				5,5
				5,008
				3,527
				2,767
	2,071	1,101	1,291	3,941
				5,479
	1,745	1,799	1,191	5,743
	3,375	1,25	1,274	1,071
Kinetic in ms	1,623	0,797	1,164	1,228
at -40mV	3,303	1,939	1,183	2,279
	3,497	0,669	1,523	4,258
	3,127			
	1,497	1,114	1,127	4,7
	2,961	1,288	1,231	3,053
	4,141	2,859	0,961	3,593
	1,148	1,23	3,057	6,963
	0,902	0,822	1,003	5,185
Mean value	2,7	1,4	1,4	4,3
Standard derivation	1,3	0,6	0,6	2,0
Number	13	11	11	17
Root	0,4	0,2	0,2	0,5
t-test				
ctrl vs TM/b1/L-b1		0,005	0,005	0,013
TM vs b1/L-b1			0,962	0,000

b1 vs L-b1	0,000



7 Special thanks to

I want to say thanks to Prof. S. H. Heinemann for this practical and Ms. Rossner and all employees and students for care, patience and help.