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Detection and classification of neurotoxins using a novel short-term plasticity quantification method

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Abstract

A tissue-based biosensor is described for screening chemical compounds that rapidly affect the nervous system. The proposed sensor is an extension of a previous work on cultured hippocampal slices [Biosens. Bioelectron. 16 (2001) 491]. The detection of the chemical compounds is based on a novel quantification method of short-term plasticity (STP) of the CA1 system in acute hippocampal slices, using random electrical impulse sequences as inputs and population spike (PS) amplitudes as outputs. STP is quantified by the first and the second order kernels using a variant of the Volterra modeling approach. This approach is more specific and time-efficient than the conventional paired pulse and fixed frequency train methods [J. Neurosci. Methods 2 (2002) 111]. Describing the functional state of the biosensor, the kernels changed accordingly as chemical compounds were added. The second order kernel was decomposed into nine Laguerre functions. The corresponding Laguerre coefficients along with the first order kernel were used as features for classification purposes. The biosensor was tested using picrotoxin (100 μM), trimethylopropane phosphate (10 μM), tetraethylammonium (4 mM), valproate (5 mM), carbachol (5 mM), DAP5 (25 μM), CNQX (3 μM), and DNQX (0.15, 1.5, 3, 5 and 10 μM). Each chemical compound gave a different feature profile corresponding to its pharmacological class. The first order kernel and the Laguerre coefficients formed the input to an artificial neural network (ANN) comprised of a single layer of perceptrons. The ANN was able to classify each tested compound into its respective class.

Keywords: CA1; Kernels; Nonlinear analysis; Paired pulse; Random train; Short-term plasticity

1. Introduction

Detection and characterization of chemical compounds that affect the nervous system have potential applications in the industrial and the military fields to identify hazardous compounds that could be harmful to civilian population and to military personnel. The major disadvantage of the current sensors is their limited ability to differentiate between various classes of chemical compounds. In order to test for a variety of compounds, different calibrated apparatuses are needed and unknown chemical compounds may be missed. Therefore, there is a need to develop a new type of sensor that can detect newly uncharacterized chemical compounds. In this article, we propose a screening biosensor based on the quantification of short-term plasticity (STP) of the CA1 hippocampal system in vitro using a newly developed approach.

The hippocampal slice with its preserved tri-synaptic pathway (Andersen et al., 1971) has been a widely used experimental preparation for investigating memory mechanisms and drug effects, using long-term potentiation (LTP) and STP (Bliss and Lomo, 1973; Alger and Teyler, 1976; Fountain and Teyler, 1995; Xie et al., 1997; Buonomano, 1999, 2000). STP based methods are typically chosen to assess drug effects of rapidly acting

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chemical compounds since they require less experimental time.

Current experimental methods for measuring STP are mostly based on the analysis of paired pulse (Dobrunz et al., 1997; Leung and Fu, 1994; Creager et al., 1980) or fixed frequency train stimulation (Papatheodoropoulos and Kostopoulos, 2000; Buonomano, 1999; Pananceau et al., 1998; Castro-Alamancos and Connors, 1997). These methods lack the ability to test all the possible time intervals in a time efficient manner. Based on a variant of the Volterra modeling approach, we have developed and implemented an efficient method to characterize and quantify STP using Poisson distributed random train (RIT) stimulation (Gholmieh et al., 2002). It can be viewed as a hybrid between the paired pulse and the fixed frequency train approaches. However, it is more time efficient than both of them. Responses to a large variety of interimpulse intervals can be evaluated in 3 min in contrast to several hours when using the paired pulse approach (Gholmieh et al., 2001, 2002). The computed STP descriptors of the underlying nonlinear dynamics were the first and second order kernels that describe the state of the system. The second order kernel was further linearly decomposed into nine Laguerre functions. The first order kernel and the coefficients of the Laguerre expansion of the second order kernel were used as features for the classification of chemical compounds.

The proposed biosensor is an extension of a previously introduced tissue-based biosensor for detecting compounds affecting cognitive function (Gholmieh et al., 2001). The biosensor consisted of hippocampal slices cultured over a multielectrode array. The current biosensor was tested using a more extensive list of chemical compounds consisting of six different classes: $GABA_A$ receptor antagonists (picrotoxin and TMPP), cholinergic agonists (carbachol), potassium channel blockers (TEA), NMDA receptor antagonists (DAP5), AMPA receptor antagonists (DNQX and CNQX), and antiepileptic drugs-Na^+ and Ca^{++} channel blockers (valproate). Each class gave a different feature profile. The first order kernel and the Laguerre coefficients formed the input to a artificial neural network (ANN) comprised of a single layer of perceptrons. The ANN was able to classify each tested compound into its respective class.

The article is organized into three sections: (1) Materials and Methods, that describes the experimental setup, the data analysis approach, and the classification algorithm; (2) Results, that presents the changes induced by the chemical compounds on the Volterra kernels (and the Laguerre coefficients), and the outcome of the classification method; (3) Discussion, that concludes the article by summarizing the advantages of the new biosensor and comparing the obtained results with those reported in the paired-pulse literature.

2. Materials and methods

2.1. Hardware materials

Electrophysiological recordings were conducted using a multimicroelectrode plate (MMEP) setup (Gholmieh et al., 2001). It consisted of a 64-electrode array (Gross et al., 1993; Univ. North Texas, http://www.cnns.org), pre-amp, data acquisition boards, and custom-designed software. The gold plated electrode Indium-Tin Oxide tips were arranged into an 8×8 formation (MMEP-4 design) with an inter-electrode distance of 150 μm.

The signals were amplified $\times 2500$ with the help of custom-built Plexon preamplifiers (http://www.plexoninc.com). Two synchronized data acquisition boards (Microstar; DAP 3200/214e series) were installed in parallel in a Pentium II 450 MHz personal computer and were able to handle 32 analog inputs with a sampling rate of 7.35 kHz. The recorded responses were evoked population spike (PS) field potential waveforms in the range of [200–1500 μV]. Stimulation was applied through an analog output channel of one data acquisition board to a Master-8 timing box (A.M.P.I). The triggered Master-8 then sent two 5 V pulses, 50 μs apart, to two stimulation boxes (Iso Flex, A.M.P.I.) connected in parallel and opposite polarity to a twisted Nichrome electrode. The software for data recording, preprocessing, and on-line analysis was based on Matlab.

2.2. Hippocampal slice preparation

Halothane anesthetized adult rats were decapitated. The hippocampus was extracted and bathed in iced aCSF. Transverse slices (thickness 400–500 μm) were collected using a Leika vibratome (VT 1000S) and were left for 2 h in aCSF to recover at room temperature. The composition of aCSF was: NaCl, 128 mM; KCl, 2.5 mM; NaH_2PO_4, 1.25 mM; $NaHCO_3$, 26 mM; Glucose, 10 mM; $MgSO_4$, 2 mM; ascorbic acid, 2 mM; $CaCl_2$, 2 mM; aerated with 95% O_2, 5% CO_2. During the recording phase, slices were perfused with aCSF at room temperature (same composition as above but with the $MgSO_4$ concentration modified to 1 mM) and were maintained submerged in the multimicroelectrode plate chamber using a nylon mesh (Fig. 1). Slices were left for 15 min to recover from manual handling. The Schaffer Collaterals were stimulated in the range of [200–400 μA] using a bipolar electrode of twisted nichrome wires (biphasic current).

2.3. Drug screening protocol

Once the stimulus intensity was set to produce 50–150% peak facilitation in the second order kernel, one random train consisting of 400 fixed amplitude impulses

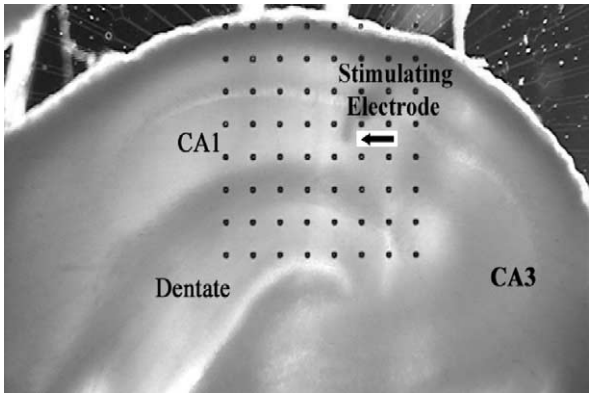

Fig. 1. Acute rat hippocampal slice positioned on the MMEP array.

with Poisson distributed interimpulse intervals (mean frequency of 2 Hz) was delivered to the Schaffer collaterals using the Nichrome electrode. After bathing the slice for 10 min with the chemical compound of interest, another random train of electrical impulses was sent. Nine sets of experiments were conducted. One set was a negative control. The other sets included: picrotoxin (100 μM), TMPP (10 μM), TEA (4 mM), valproate (5 mM), carbachol (5 mM), DAP5 (25 μM), CNQX (3 μM), and DNQX. The DNQX set was further divided into five subsets of different concentrations: 0.15, 1.5, 3, 5, and 10 μM.

2.4. Analytical methods

The data was analyzed using the Volterra modeling approach adapted for random interimpulse interval stimuli sequences and PS amplitude response sequence (Courellis et al., 2000; Gholmieh et al., 2002). A second order model was used, expressed by the following equation:

$$y(n_i) = k_1 + \sum_{n_i-\mu<n_j<n_i} k_2(n_i - n_j) \quad (1)$$

where, n_i is the time of occurrence of the i-th stimulus impulse, n_j is the time of occurrence of the j-th stimulus impulse prior to the i-th stimulus impulse, $y(n_i)$ is the amplitude of the PS response to the i-th stimulus impulse, μ is the memory of the biological system, k_1 is the first order kernel, and k_2 is the second order kernel. The first order kernel represents the mean PS amplitude, while the second order kernel quantifies the effect on the current PS amplitude of the interaction between the current stimulus impulse and each past stimulus impulse within the memory window μ. The second order kernel can be further decomposed into a linear combination of L Laguerre functions (Marmarelis, 1993) in the form of:

$$k_2(n_i - n_j) = \sum_{l=0}^{L-1} c_l L_l(n_i - n_j) \quad (2)$$

where $L_l()$ is the l-th order Laguerre function (Fig. 2) and c_l is the corresponding Laguerre expansion coefficient. We have empirically determined that reliable representation of the CA1 *in vitro* nonlinear dynamics required nine Laguerre functions for the expansion of the second order kernels (Gholmieh et al., 2002). The nine Laguerre coefficients were normalized to the peak facilitation. The normalized coefficients along with the first order kernel were used for chemical compound detection and profiling.

2.5. Classification method

The classification method was divided into two steps (Fig. 3). The first step divided the chemical compounds into three preliminary classes depending on their effect on k_1: inhibitory (a decrease in the value of k_1), excitatory (an increase in the value of k_1), or no effect (no change in the value of k_1). The second step was used to classify the chemical compounds into more specific classes. It was formed by a single layer perceptron ANNs using the Laguerre coefficients as input. A hardlimiter was used as the activation function, causing the neuron output to be either one or zero. Seven out of the nine experimental sets (excluding TMPP and CNQX) were used to train the classifier. TMPP and CNQX were used as test cases (outside the training set) to evaluate the classifier.

3. Results

Nine sets of experiments, each including five to seven experiments, were conducted. The nine sets included one negative control and eight chemical compounds that belong to six different pharmacological classes. In particular, we used picrotoxin (100 μM), valproate (5 mM), tetraethylammonium (4 mM), carbachol (5 mM), DAP5 (25 μM), DNQX (0.15, 1.5, 3, 5 and 10 μM), TMPP (10 μM), and CNQX (3 μM). The first seven sets were used to train the ANNs, while the last two sets (i.e. TMPP and CNQX) we utilized to test the classification ability of the neural network.

The effect of various chemical compounds on the first order kernel (k_1) was quantified as the ratio of post-drug k_1 value to pre-drug k_1 value (Fig. 4A). A chemical compound will cause an increase or a decrease in k_1, if the ratio is greater or less than one, respectively. Picrotoxin, TMPP, and TEA caused an increase in the k_1 ratio, while DNQX, CNQX, DAP5, carbachol, and valproate caused a decrease in the k_1 ratio. No change in the k_1 ratio was seen in the negative control set.

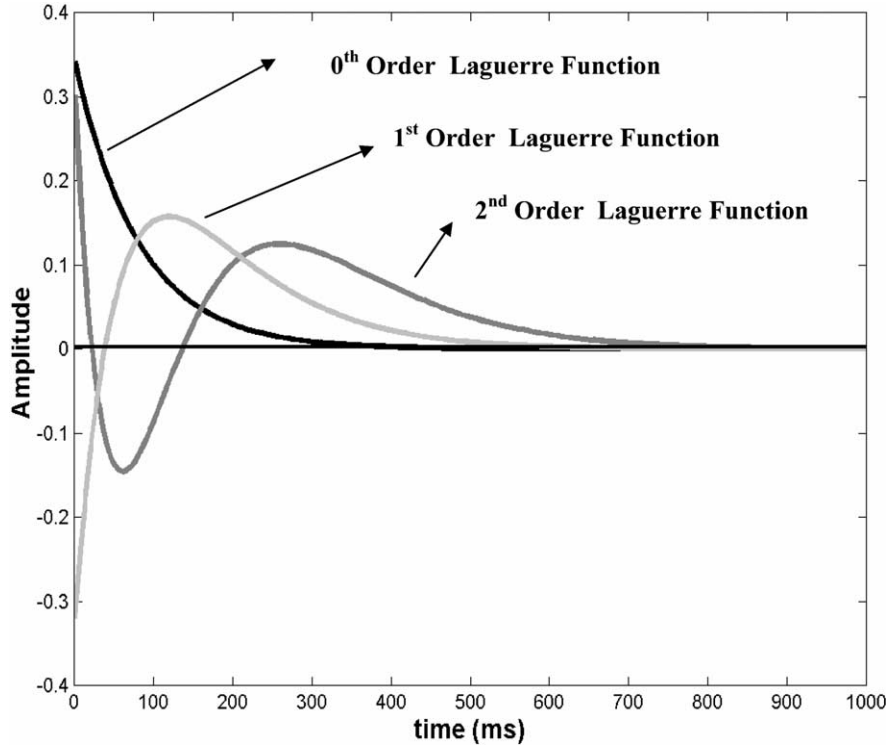

Fig. 2. The first, second, and third Laguerre functions.

The negative control study is summarized in Fig. 4B. The six conducted experiments showed very low variability in the first and second order kernels (Fig. 4B) since there was no statistically significant change in the value of any of the Laguerre coefficients (inset) or in the value of the first order kernel ($P < 0.01$). Moreover, the

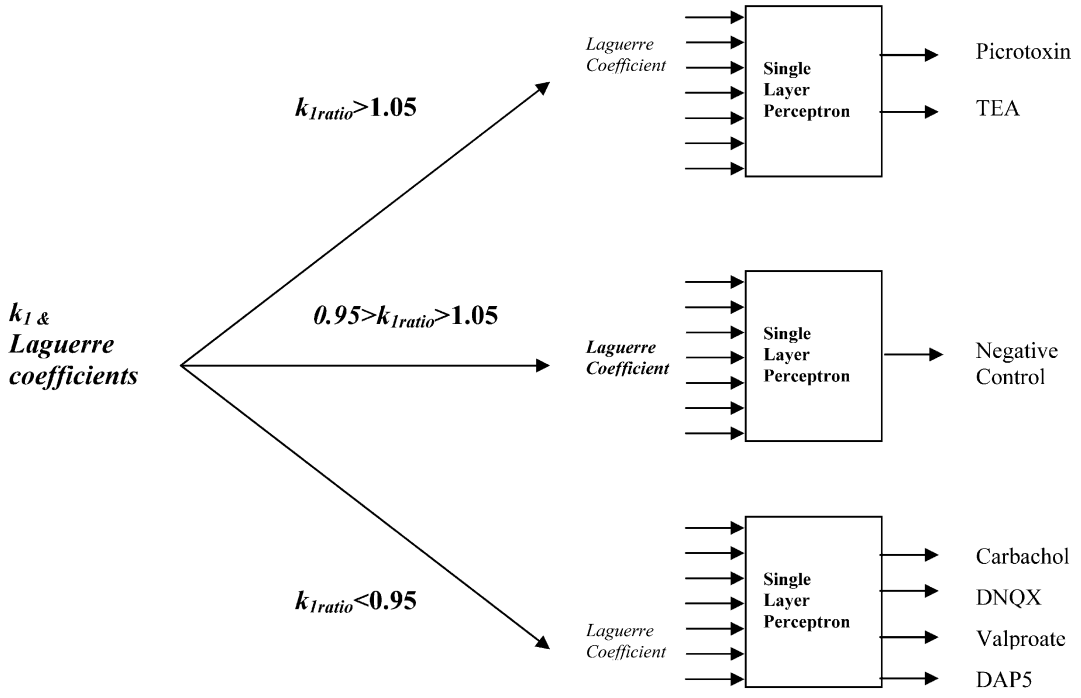

Fig. 3. The classification algorithm. The first order kernel ratio along with the Laguerre coefficients formed the input to the classifier. The compound was first classified through its ability to increase or decrease the k_1 ratio. In the second stage, the Laguerre coefficients formed the input to a single layer of perceptrons that categorized each compound into its specific class. Only seven out of nine coefficients were used for classification (shown by the F-ratio analysis in Section 3).

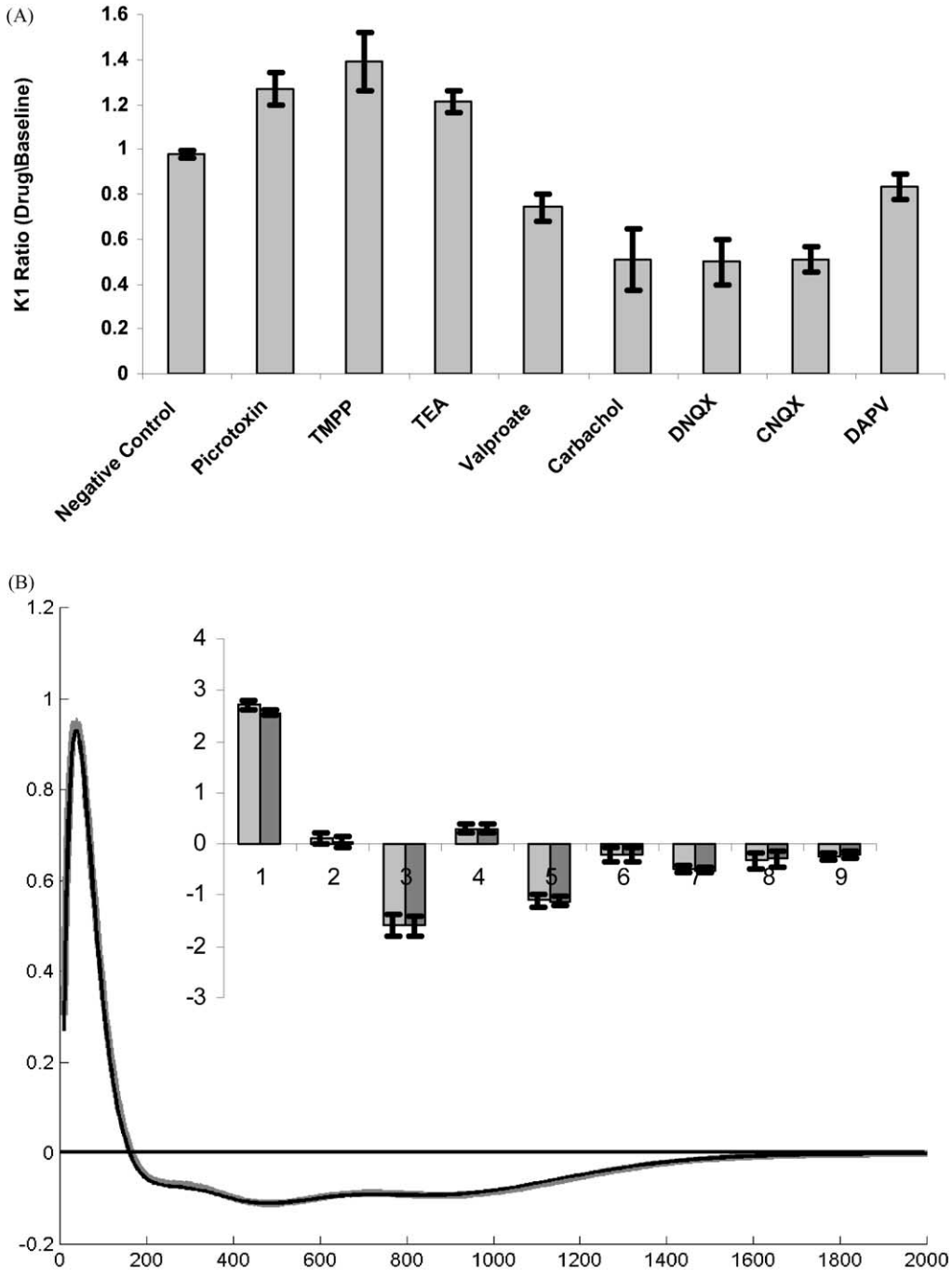

Fig. 4. (A) Effect of various chemical compounds on the k_1 ratio. (B) Negative control study. Second order kernel before (gray) and after (black) addition of a negative control solution. As expected there was no change in the STP properties under negative control testing conditions. Inset: Effect of negative control on the Laguerre coefficients (baseline: gray; negative control: dark gray; bars: ± 1 S.D.).

second order kernel exhibited the following characteristics under control conditions: (1) a facilitation peak between 25 and 45 ms; (2) a fast rising phase [0–30 ms] before the peak; (3) a fast facilitatory relaxation phase [30–200 ms]; and (4) an inhibitory phase starting at 200 ms and returning to the baseline within 1600–2000 ms.

Picrotoxin ($n = 5$) caused an increase in the value of the first order kernel by 27% (S.D. $\pm$15%), and an increase in peak facilitation value of k_2 by 75%. The picrotoxin effect on STP (Fig. 5A) caused a significant increase in the value of the first ($P < 0.01$), third ($P < 0.01$), fourth ($P < 0.01$), fifth ($P < 0.05$), and ninth ($P < 0.05$) Laguerre coefficient (inset).

Trimethylpropane phosphate ($n = 6$) increased k_1 value by 39% (S.D. $\pm$13%), k_2 peak facilitation by 30%, and the amplitude of the inhibitory phase (Fig.

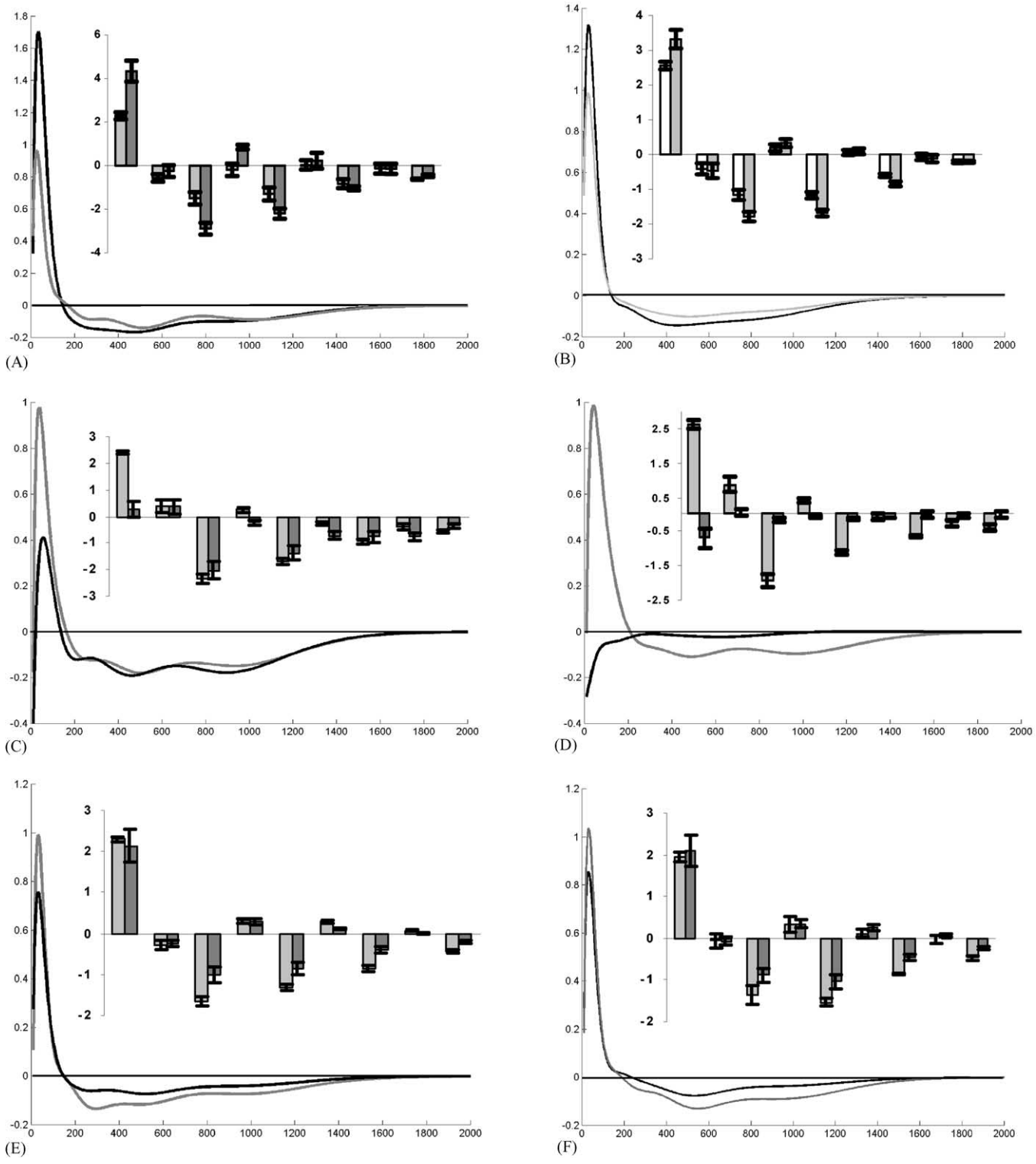

Fig. 5. Effect of various chemical compounds on the second order kernel and the corresponding Laguerre coefficients. Baseline second order kernel and the corresponding Laguerre coefficients (Inset): shown in gray. Chemical compound effect on the second order kernel shown in black. The effect of the chemical compound on the Laguerre coefficients shown in dark gray. (A) Picrotoxin. (B) TMPP. (C) Tetraethylammonium. (D) Carbachol. (E) DNQX. (F) CNQX.

5B). TMPP also caused a significant increase in the value of the first ($P < 0.05$), third ($P < 0.05$), fifth ($P < 0.01$), and seventh ($P < 0.05$) Laguerre coefficient (inset).

TEA ($n = 6$) at 4 mM increased the value of k_1 by 21% (S.D. $\pm$11%), decreased the peak facilitation value of k_2 by 60%, increased the early inhibitory phase [5–15 ms] and the area of the late inhibitory phase [200–1200 ms] (Fig. 5C). TEA also caused statistically significant change in the value of the first ($P < 0.01$), fourth ($P < 0.01$), sixth ($P < 0.05$) and ninth ($P < 0.05$) Laguerre coefficient (inset).

Carbachol ($n = 6$) at 5 mM caused the STP properties of CA1 to shift from facilitation to depression. In addition to causing the k_1 value to drop by 49% (S.D. $\pm$36%), carbachol caused the second order kernel to become a negative exponential decaying function with peak inhibition value of -30% (Fig. 5D). The effect is reflected in a statistically significant manner in the Laguerre coefficients (inset). In particular, the value of the first and fourth coefficient became negative, and the value of the second, third, fifth, and seventh Laguerre coefficient was markedly decreased ($P < 0.01$).

DNQX at 3 μM ($n = 6$) caused a decrease in the value of the first order kernel by 49% (S.D. $\pm$24%) and in k_2 peak facilitation value by 25% (Fig. 5E). The effect of DNQX was further characterized through the changes in the Laguerre Coefficients. Variations were observed in the value of the third ($P < 0.01$), fifth ($P < 0.05$), seventh ($P < 0.01$), and ninth ($P < 0.01$) Laguerre coefficient (inset). No significant change was seen in the value of the first coefficient.

CNQX ($n = 5$) caused a decrease in the value of the first order kernel by 50% (S.D. $\pm$12%) and in the k_2 peak facilitation value by 15% (Fig. 5F). Like DNQX, no significant change was seen in the value of the first coefficient, but a decrease was observed in the value of the third, fifth ($P < 0.05$), seventh ($P < 0.01$), and ninth ($P < 0.01$) coefficient.

Valproate ($n = 6$) decreased the k_1 value by 26% (S.D. $\pm$0.16) and increased the k_2 peak facilitation value by 20% (Fig. 6A). The effect on STP decreased the value of only the first Laguerre coefficient ($P < 0.05$).

DAP5 ($n = 7$) decreased the k_1 value by 17% (S.D. $\pm$ 0.15) and the k_2 peak facilitation value by 10% (Fig. 6B). However, DAP5 did not cause any statistically significant changes in most of the Laguerre coefficients (inset) except for decreasing the value of the second coefficient ($P < 0.05$).

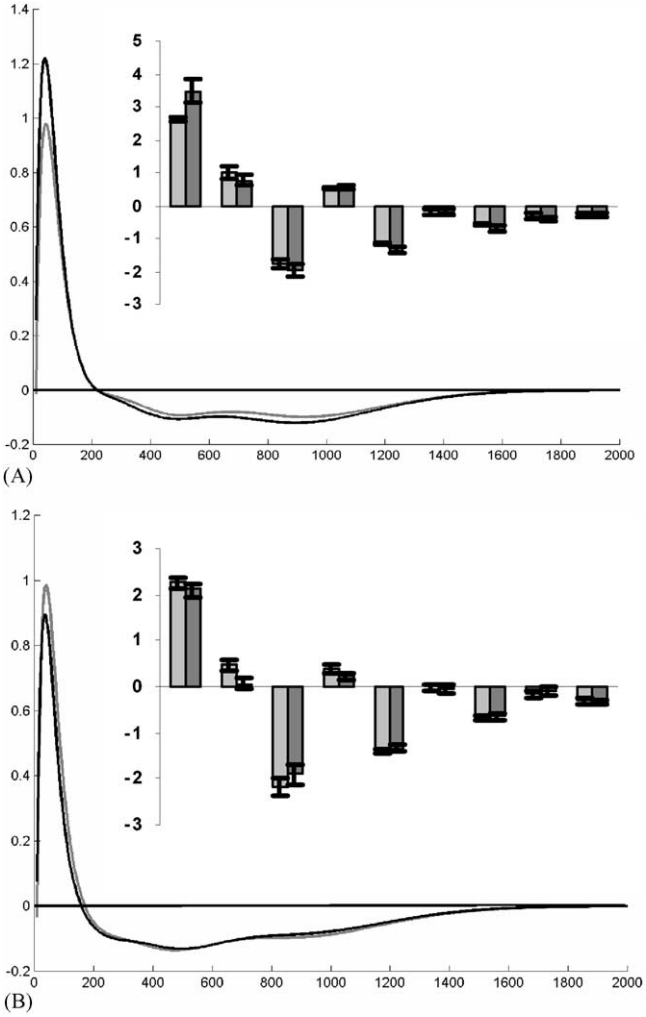

Fig. 6. Effect of DAP5 and valproate on the second order kernels and the corresponding Laguerre coefficients. Baseline second order kernel and the corresponding Laguerre coefficients (Inset) shown in gray. Chemical compound effect on the second order kernel shown in black. The effect of the chemical compound on the Laguerre coefficients shown in dark gray. (A) Valproate. (B) DAP5.

3.1. DNQX results

DNQX was tested at concentrations of 0.15, 1.5, 3, 5 and 10 μM. The choice of concentrations was based on the current literature where 10 μM is known to block AMPA receptors completely while 3 μM is known to cause 50% decrease in the field potential amplitude (i.e. IC50). As expected, DNQX at 10 μM abolished the first and the second order kernel (Fig. 7A). The k_1 ratio decreased in a dose-dependent manner, i.e. the k_1 ratios were 0.99, 0.91, 0.51, 0.41, and 0 for the concentrations of 0.15, 1.5, 3, 5 and 10 μM, respectively. There was a dose-dependent decrease in the peak facilitation and the late inhibitory phase. Interestingly, there was also a dose-dependent decrease in the value of the third, fifth, and seventh Laguerre coefficient (Fig. 7B).

3.2. Classification results

As mentioned in Section 2, the classification algorithm consisted of two steps (Fig. 3). The first step classified the compounds using k_1 ratio. TEA and Picrotoxin caused an increase in k_1 and were classified

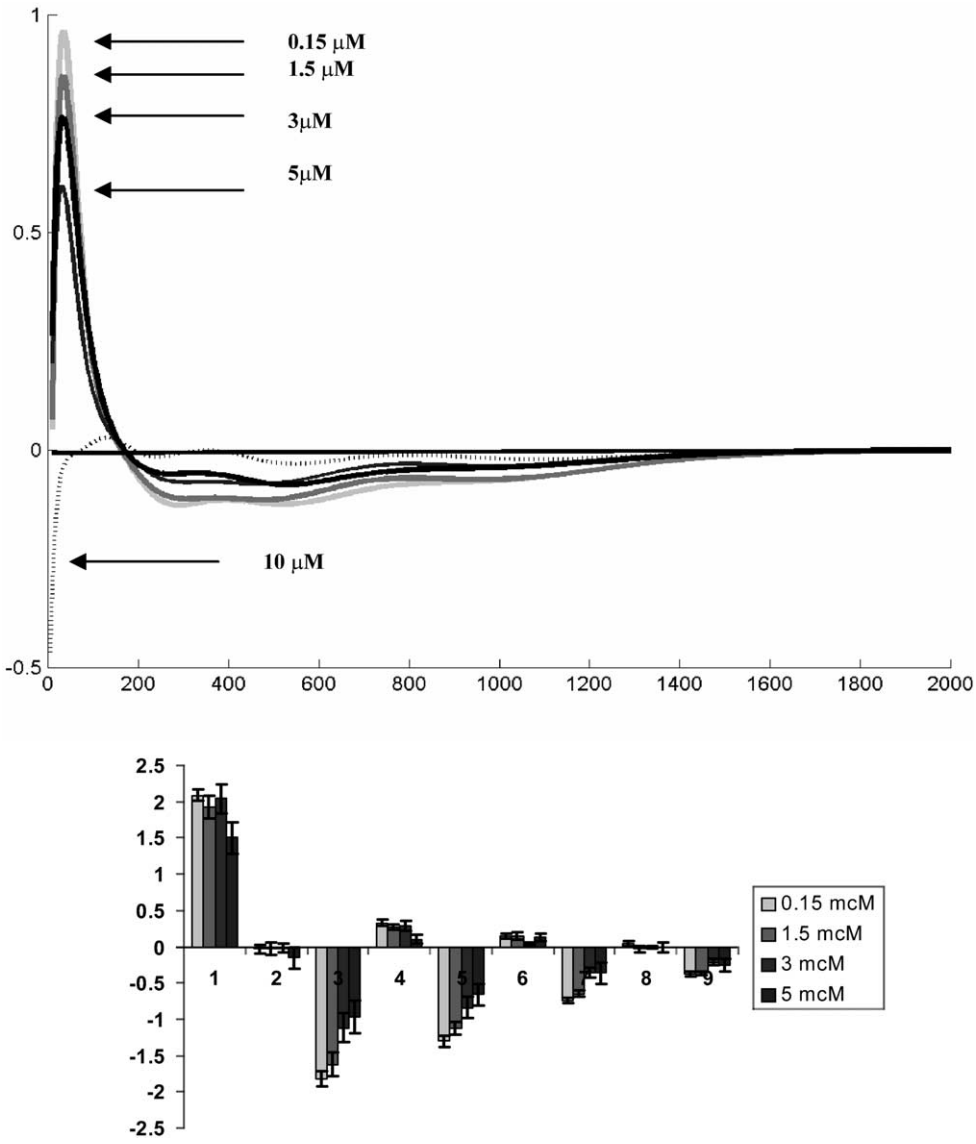

Fig. 7. DNQX Effect on STP. (A) Dose-dependent decrease in the peak facilitation and the late inhibitory phase of the second order kernel. (B) Dose-dependent decrease in the value of the third, fifth, and seventh Laguerre coefficient.

in the first preliminary class. The negative control caused no change in the k_1 ratio and formed the second preliminary class. Valproate, DNQX, DAP5 and carbachol formed the third preliminary class through their depressive effect on k_1. The second step of the classification algorithm consisted of feeding the Laguerre coefficient into the ANN.

In order to determine which coefficients were suitable for the second classification step, we used one-way ANOVA to analyze the pre-drug baseline among the different experimental sets. Statistical analysis of the pre-drug baseline parameters indicated that the first order kernel and the first, third, fourth, fifth, sixth, seventh, and eight Laguerre coefficient were sampled from the same population (Table 1, $F_{\text{critical}} < 3.35$). The inset in Fig. 8 shows the averaged coefficients across the different experimental sets with the corresponding second order kernel shown in the background.

Since the second and the ninth coefficients were statistically non-significant, the number of inputs to the neural network was reduced to seven. Overall the ANN was able to classify 81% of the forty-three training experiments. The results were as follows: picrotoxin (4/5), TEA (5/6), negative control (6/7), valproate (5/6), carbachol (5/6), DAP5 (5/7), and DNQX (4/6). The trained neural network classified the 1.5, 3 and 5 μM sets of DNQX correctly, and the 0.15 μM set of DNQX as negative control. The latter result was anticipated since DNQX at a concentration of 0.15 μM did not affect k_1 or k_2 (Fig. 7A). The classifier also classified TMPP in the same class as picrotoxin and CNQX in the same class as DNQX. This result was expected, since

Table 1
Mean values and the standard deviations of the first order kernel and the nine Laguerre coefficients of the second order kernel for each set of experiments before adding the chemical compound

	Control	Picrotoxin	Valproate	TEA	Carbachol	DNQX	DAP5	*F*-value
N	7	5	6	6	6	6	7	
k_1 (μV)	484 (±137)	399 (±126)	248 (±38)	250.13 (±49)	391 (±220)	329 (±180)	346 (±160)	2.10
Coeff 1	2.71 (±0.21)	2.28 (±0.36)	2.65 (±0.21)	2.40 (±0.15)	2.63 (±0.32)	2.30 (±0.23)	2.25 (±0.31)	3.31
Coeff 2	0.11 (±0.26)	−0.56 (±0.38)	0.98 (±0.49)	0.37 (±0.59)	0.87 (±0.49)	−0.28 (±0.41)	0.47 (±0.30)	6.33
Coeff 3	−1.59 (±0.53)	−1.50 (±0.63)	−1.84 (±0.30)	−2.34 (±0.38)	−1.92 (±0.46)	−1.65 (±0.43)	−2.17 (±0.52)	2.59
Coeff 4	0.30 (±0.27)	−0.19 (±0.64)	0.4 (±0.28)	0.24 (±0.20)	0.38 (±0.15)	0.32 (±0.22)	0.38 (±0.24)	2.53
Coeff 5	−1.10 (±0.32)	−1.30 (±0.66)	−1.18 (±0.10)	−1.70 (±0.26)	−1.08 (±0.15)	−1.30 (±0.26)	−1.39 (±0.16)	2.56
Coeff 6	−0.21 (±0.37)	0.03 (±0.46)	−0.20 (±0.20)	−0.25 (±0.26)	−0.07 (±0.21)	0.28 (±0.17)	−0.01 (±0.20)	2.80
Coeff 7	−0.49 (±0.15)	−0.83 (±0.44)	−0.58 (±0.11)	−0.98 (±0.16)	−0.64 (±0.11)	−0.85 (±0.26)	−0.66 (±0.16)	3.15
Coeff 8	−0.32 (±0.41)	−0.13 (±0.51)	−0.31 (±0.23)	−0.37 (±0.27)	−0.25 (±0.24)	−0.07 (±0.13)	−0.16 (±0.24)	1.42
Coeff 9	−0.23 (±0.18)	−0.61 (±0.05)	−0.25 (±0.23)	−0.58 (±0.14)	−0.39 (±0.19)	−0.44 (±0.14)	−0.31 (±0.13)	5.17

N represents the number of experiments performed for each chemical compound.

TMPPP and picrotoxin belong to the same pharmacological class, and CNQX and DNQX also belong to the same pharmacological class.

4. Discussion

A screening tool for classifying chemical compounds that affect the nervous system has been introduced. It is based on a novel method for analyzing STP of the CA1 hippocampal region in vitro. It is an extension of a previously described tissue-based biosensor for detecting compounds affecting cognitive function (Gholmieh et al., 2001). The proposed analytical approach uses the first order kernel and the Laguerre coefficients of the second order kernel as features for classification.

Using the proposed biosensor, the effects of the following classes of chemical compounds were analyzed: $GABA_A$ receptor antagonists (picrotoxin and TMPP), cholinergic agonists (carbachol), potassium channel blockers (TEA), NMDA receptor antagonists (DAP5), AMPA receptor antagonists (DNQX and CNQX), and antiepileptic drugs- Na^+ and Ca^{++} channel blockers (valproate). All these chemical compounds produced specific alterations in the value of the first order kernel and the Laguerre coefficients of the second order kernel.

We compared the results obtained using our approach to those obtained in the literature using the paired pulse approach. Based on our recent work (Gholmieh et al.,

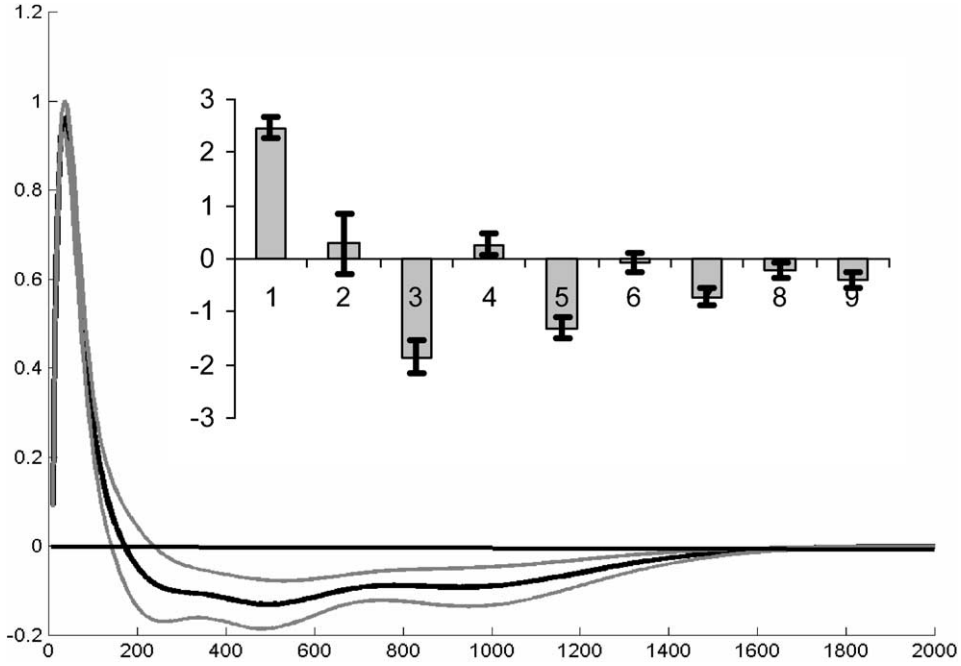

Fig. 8. Average of the second order kernel (black) across the various experimental set baselines (pre-drug). The two gray lines delimit one standard deviation. Inset: Average of the corresponding Laguerre coefficients.

2002), changes in the amplitude of the conditioning response were compared with changes in the value of k_1, and changes in the amplitude of the test response were compared with changes in the value $k_1+k_{2\ max}$, since the interimpulse interval chosen for testing chemical compounds in most paired pulse studies usually corresponds to the maximum possible facilitation.

$GABA_A$ inhibitors like picrotoxin and bicuculline are well known for their ability to inhibit early postsynaptic inhibitory potential (Davies et al., 1990; Lacaille, 1991). We have found that picrotoxin (100 μM) increased the value of the first order kernel by about 21%, and increased the peak facilitation value of the second order kernel by 70% (Fig. 5A). These results are consistent with previous findings that $GABA_A$ antagonist increased the PS amplitude of both the conditioning and test response in paired pulse studies (Steffensen and Henriksen, 1991; Leung and Fu, 1994).

Several recent studies have shown that TMPP induces epileptiform activity in CA1 (Lin et al., 1998, 2001) by acting as a $GABA_A$ antagonist (Keefer et al., 2001; Kao et al., 1999; Higgins and Gardier, 1990). Our results confirm earlier reports by showing that TMPP increased CA1 excitability by augmenting the value of the first order kernel, the second order kernel, and the Laguerre coefficients in a similar manner to picrotoxin (Fig. 5A, B).

TEA was used in a concentration of 4 mM since 12.5 and 25 mM caused seizure activity in preliminary experiments. We observed an increase in the value of k_1 that is consistent with previous articles reporting that TEA caused an increase in the CA1 field responses (Southan and Owen, 1997; Song et al., 2001). In addition, the second order kernel showed an increase in the early inhibitory phase and a decrease in the peak facilitation value of k_2 (Fig. 5C).

Carbachol, a cholinergic agonist, caused a suppression of the value of k_1 and a dramatic shift of the second order kernel from facilitation to depression (Fig. 5D). The depressive effect on k_1 and k_2 is consistent with previous reports of dose dependent field potential suppression (Hesen et al., 1998; Yajeya et al., 2000).

AMPA receptors are thought to mediate the bulk component of field potentials at the CA1 glutamatergic synapses. DNQX and CNQX are well known for being AMPA receptor antagonists (Andreasen et al., 1989). Both compounds had no effect on the first coefficient and caused a depressive effect on the third, fifth, and seventh coefficient. The peak facilitation value of k_2 for DNQX and CNQX decreased by 23 and 14%, respectively (Fig. 5E, F) without showing statistically significant difference ($P<0.05$). We also observed dose-dependent DNQX suppression of the first and second order kernels (Fig. 7). The IC50 of DNQX on k_1 was found to be around 3 μM (consistent with a previous report (Andreasen et al., 1989)) while the IC50 for the second order kernel peak facilitation value was around 5 μM.

Valproate is anti-seizure medication that is known to block Ca^{++} and Na^{+} channels. We have observed a decrease in k_1 value with a contrasting moderate increase in k_2 peak facilitation value, and an increase in the value of only the first Laguerre coefficient. A previous paired pulse study showed a decrease in the conditioning response (Franceschetti et al., 1986) consistent with the depressive effect of valproate on k_1. The effect of valproate on k_1 and k_2 partially mimicked the effects caused by low calcium concentration. Low calcium concentration in the aCSF caused a decrease in the value of k_1 and an increase in the value of the first Laguerre coefficient (unpublished observation). Previous IO and paired pulse studies showed that lowering calcium concentration decreased the conditioning response by shifting the IO curve to the right and downward (Stringer and Lothman, 1988) and increased the amplitude and shifted the stimulus-response curve of the conditioned response to the left (Sagratella et al., 1991; Igelmund and Heinemann, 1995). These observations are consistent with the calcium channel blocking effect of valproate.

DAP5 is an NMDA receptor antagonist. In our study, DAP5 caused a decrease in the k_1 value by 17% and in the peak facilitation value of k_2 by 10%. The modest effect of DAP5 on the STP descriptors is due to the relatively high concentration of magnesium in the aCSF and the low mean frequency of stimulation. Our results are consistent with a previous study (Muller and Lynch, 1990) suggesting that the NMDA component of the conditioning EPSP at 1 mM of Mg was around 10% and that the peak facilitation of the test EPSP response decreased by 10% upon the addition of the NMDA channel blocker.

The results of our study showed that each class of compounds caused specific changes in the first order kernel and the Laguerre coefficients of the second order kernel. These features formed the basis for classification using ANNs. The classifying ANN used a single layer perceptrons and was able to classify each chemical compound into its respective class. The ANN classified the 0.15 μM DNQX set as negative control. The latter result was expected since the 0.15 μM did not cause any effect on the first and the second order kernels. More interestingly, the trained neural network classified correctly compounds outside the training sets, e.g. TMPP and CNQX, into their respective pharmacological class.

In conclusion, we have demonstrated that different classes of chemical compounds can be successfully classified according to their effect on the first order kernel and the Laguerre coefficients of second order kernels describing STP at the CA1 hippocampal region in vitro. Although our study assessed the immediate

effect of the chemical compounds, the delayed effect of each compound can also be explored by reapplying the RIT sequence and computing the kernels every 10 min or by using an ex-vivo slice approach, wherein an animal is exposed to the toxicant in advance of slice preparation.

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