行政院國家科學委員會補助專題研究計畫成果報告

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※年齡影響長程促進作用與長程抑制作用其機制之探討※
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計畫類別：☑個別型計畫　　☐整合型計畫

計畫編號：NSC　－89- 2314- B002- 060

執行期間：88 年　08 月　01日至 89　年　07 月　31 日

計畫主持人：沈友仁

共同主持人：

本成果報告包括以下應繳交之附件：

☐赴國外出差或研習心得報告一份

☐赴大陸地區出差或研習心得報告一份

☐出席國際學術會議心得報告及發表之論文各一份

☐國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學醫學院小兒科

中　華　民　國　89　年　10　月　26　日

行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC-89-2341-B002-060

年齡影響長程促進作用與長程抑制作用其機制之探討，第一年計畫

執行期限：88年8月1日至89年7月31日

主持人：沈友仁　國立台灣大學醫學院小兒科

計畫參與人員：楊千立 國立台灣大學醫學院小兒科

中文摘要：

科學家們相信，學習與記憶的形成，與突觸傳導的強化與抑制有關，稱之為神經突觸的可塑性(synaptic plasticity)。若突觸前神經受到高頻持續性刺激(tetanus)之後，再給予原來同樣強度的刺激，則此時所產生的EPSP會比原來的更大。這樣的強化效果，可以持續數小時甚至數天之久，稱之為長程強化效應(long-term potentiation, LTP)。如果神經細胞受到低頻率(1 Hz, 15 分鐘)的刺激(low frequency stimulation)，之後若再給予原來強度的刺激，則所產生的EPSP將會比原來的小。這樣的反應也會持續數小時之久，稱之為長程抑制效應(long-term depression, LTD) 。我們發現，在幼鼠（2至3週大）海馬回腦切片CA1區域每一片皆可有效的誘發長程抑制效應(Long-term depression, LTD)。相對的，低頻刺激在較成熟的鼠腦便不易產生LTD。至於高頻刺激，腦切片經過tetanus之後， 64% (7/11) 2週大的切片產生LTP。在4週大的切片中，82% 產生LTP，這些產生LTP的細胞反應平均起來，其population spike (PS)是基礎反應的231±25% (n=9)。在8週大的切片裡，57%產生LTP，其PS是基礎反應的150±17% (n=8)。我們進而探討不同年齡鼠腦海馬回的NMDA反應是否有不同。NMDA反應是用無鎂ACSF灌流，再以CNQX及bicuculline抑制AMPA及GABA反應而得。結果發現，NMDA反應與突觸前反應之大小有關，若以此兩者之關係做曲線，可以發現兩曲線顯著不同，幼鼠曲線Bmax為22.76±2.89, Kd0.305±0.100, 而成鼠之Bmax 為101.3±5.94, (p<0.0001), Kd為0.699±0.08，p=0.0048有統計學上之差異。

關鍵詞：年齡、突觸傳導、NMDA, 長程強化效應、長程抑制效應

Abstract

LTP and LTD are currently believed to be the cellular models for studying learning and memory. In neonatal rats (2 weeks old), LTD was routinely produced by LFS of 900 pulses at 1 Hz. On the other hand, LTD could not be routinely induced by LFS in juvenile (4-5 weeks old) and adult rats (8 weeks old). As for long term potentiation, 7 of 11 hippocampal slices from 2-week-old rats developed LTP after 3 trains of 100 Hz stimulation. In 4-week-old rats, 9 of 11 slices developed LTP after tetanus. In adult rats, 8 of the 14 slices tested showed LTP. There was a age-dependent change in the magnitude of LTP, with the juvenile rats showing the highest level, and the adult rats the lowest among the 3 age groups tested. We studied the NMDA response in infant rats and in adult rats. The NMDA response was induced by perfusing the slice with Mg++ free ACSF, with CNQX and bicuculline to block the response of AMPA receptor and GABA receptors. We found out that the NMDA response correlated with the size of presynaptic volley. If we plotted the NMDA response against the presynaptic volley size, we got 2 one-phase binding curve. The Bmax for the infant was 22.76±2.89, Kd 0.305±0.100. For the adult, theBmax was 101.3±5.94, ($p<0.0001$) and the Kd was 0.699±0.08，$p=0.0048$. This indicates that the NMDA response changed according to age. This change maybe one of the reasons for the different response of LTP/LTD in different ages.

Key Words: age, synaptic transmission; NMDA, LTP, LTD

Background

From previous reports in the literature, it is clear that long term potentiation (LTP) and long term depression (LTD) are age-dependent. In the hippocampal CA1 region of rat brain, LTD is readily inducible by 2 week of age, became difficut to be induced in the adult and

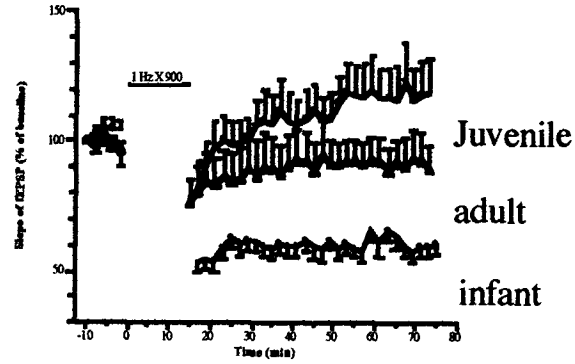
inducible again in the aged-rats. On the other hand, LTP is difficult to be induced at neonatal stage, prominent after 4 weeks of age, saturated in the young adult stage then gradully decline. LTP is difficult to sustain in the aged-rats. Although not fully understood, the mechanism underlying the formation of LTD and LTP in CA1 region are believed to be dependent on NMDA receptor. Ater NMDA receptors are activated, with moderate elevation of intracellular Ca2+, calcineurin is activated and the post-synaptic response is depressed. With high Ca2+ influx through NMDA receptors, CAMK II is activated, and the post-synaptic response is potentiated. But why the formation of LTD and LTP is age-dependent? It has been shown that different subtypes of NMDA receptors are expressed through the development of brain, and the expression of calcineurin and CAMK II are also age dependent. In hippocampus, calcineurin is most abundent at 2 weeks of age and gradually decline. Calmodulin is relatively stationary throughout development in hippocampus. From these evidences, we can postulate several possiblilities to explain the age-dependency of synaptic plasticity. Different subtypes of NMDA receptors may have different affinity for calcium, thus, under the same stimulation protocal, the calcium influx may be different. With different levels of intracellular calcium, LTD and LTP may be easier or more difficult to be induced. We would like to know first whether the calcium infulx through NMDA receptor in the physiological situation is different at different age.

Material and Methods

Extracellular recordings were made in CA1 region of hippocampal slices obtained from male Spraque-Dawley rats of 4-5 weeks old. The rats were decapitated and the brains rapidly removed from the skull. Coronal slices of 400 μm thick were cut and the appropri-ate slices were placed in a beaker of artificial cerebrospinal fluid (ACSF). The ACSF was bubbled continuously with 95% O2-5% CO2 to maintain proper pH (7.3-7.5). A single slice was then transferred to the re-cording chamber where it was held submerged between two nylon nets and maintained at 32±1°C. Extracel-lular recordings of field excitatory postsynaptic potentials (fEPSPs) and population spikes (PSs) were obtained from stratum radiatum and stratum pyramidale, respectively, using microelectrodes filled with 3 M NaCl (3-8 MΩ). A bipolar stimulating electrode was placed in stratum radiatum. The stimulus duration was 150 μsec. To induce LTD, low frequency stimulation with 1Hz for 15 min were given. To induce LTP, 3 trains of tetani with 100Hz, doubled voltage for 1 sec were given with a train interval of 20 min. To see the NMDA response, the slices were perfused with Mg++ free ACSF containing 10μM CNQX and 20 μM bicuculline. The voltage was then increased stepwised until a maximum response. The spike area of the presynaptic volley was then plotted against the area of the NMDA resonse. The spike area of the presynaptic volley and the area of the NMDA response was analyzed with Axograph 4.1 (Axon Instrument, USA). The curves were fit with Prism 3.1, using the one-site-binding hyperbola model Y=Bmax*X/(Kd+X). t test was used to compare the difference of the parameters of these curves. A p value < 0.05 was considered as significant. All data were expressed as mean±S.E.M.

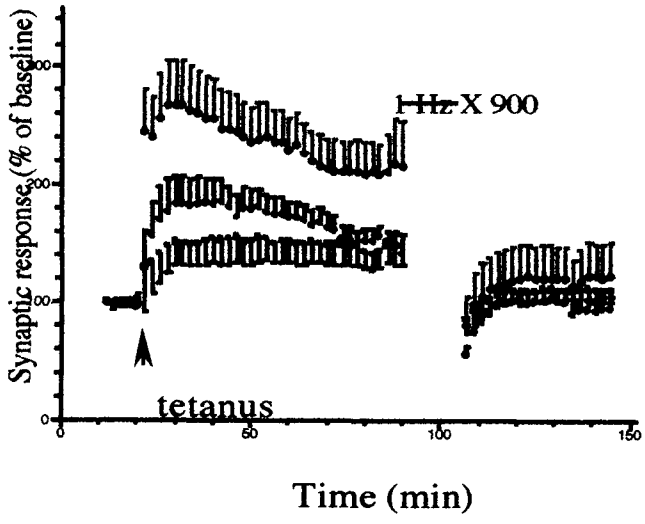
Results

In neonatal rats (2 weeks old), LTD was routinely produced by LFS of 900 pulses at 1 Hz. The fEPSP 40 min after LFS was 59±4% (n=18) of baseline response.On the other hand, LTD could not be routinely induced by LFS in juvenile (4-5 weeks old) and adult rats (8 weeks old). In juvenile rats , after LFS, fEPSPs were actually slightly potentiated, being 118±11% (n=17) of the baseline. In adults, the fEPSPs was 92±8% (n=17) of the baseline (fig.1)

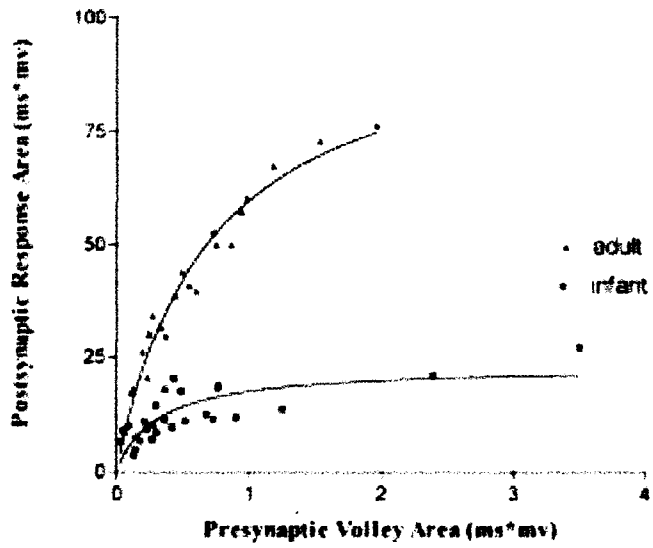


As for long term potentiation, 7 of 11 hippocampal

slices from 2-week-old rats developed LTP after 3 trains of 100 Hz stimulation. The slope of fEPSP 40 min after tetanus was 172±14% (n=7) of baseline. LFS was then delivered and the fEPSP 40 min after LFS was 56±4% of pre-LFS level. In 4-week-old rats, 9 of 11 slices developed LTP after tetanus. The amplitude of population spike 40 min after tetanus was 231±25% (n=9) of baseline. After LFS, it was 54±8% (n=9) of the pre-LFS level. In adult rats, 8 of the 14 slices tested showed LTP, being 150±17% (n=8) of baseline and the depotentiation of 79±6% (n=8). There was thus a age-dependent change in the magnitude of LTP, with the juvenile rats showing the highest level, and the adult rats the lowest among the 3 age groups tested (fig 2).



We studied the NMDA response in infant rats and in adult rats. The NMDA response was induced by perfusing the slice with Mg^{++} free ACSF, with CNQX and bicuculline to block the response of AMPA receptor and GABA receptors. We found out that the NMDA response correlated well with the size of presynaptic volley. If we plotted the NMDA response against the presynaptic volley size, we got 2 one-phase binding curve.



The Bmax for the infant was 22.76±2.89, Kd 0.305±0.100. For the adult, the Bmax was 101.3±5.94, ($p<0.0001$) and the Kd was 0.699±0.08 , $p=0.0048$. This indicates that the NMDA response changed according to age. This change maybe one of the reasons for the different response of LTP/LTD in different ages.

Discussion

Our results showed that both LTP and LTD are age dependent, but the depotentiation is not. Only in infancy can LTD readily induced. The juvenile brain showed greatest amplitude in LTP, and less likely to be induced to have LTD. This correlated well with clinical observation that the adolescents are more impulsive, excitable, and active in comparison with the young infant and mature adults. In the literature, it was also shown that LTD in not routinely inducible in the adult and the LTP not routinely inducible in the neonate. We were still able to induce LTP in the neonatal rats, probably because we used 3 trains of tetani instead of 2 trains used in other reports.

To know if such age-dependent change of synaptic plasticity related to NMDA responses, we elucidated the age-dependency of NMDA response. In CA1 region of rat hippocampus, the major postsynaptic receptors are AMPA, GABA and NMDA. In resting states, the NMDA was blocked by Mg++. By perfusing the slice with Mg++ free ACSF and

CNQX and bicuculline, we were able to isolate NMDA response in this area. We found that by increasing the stimulation voltage, the presynaptic volley increased, and the NMDA response also increased, but saturable. With the same degree of presynaptic volley, the NMDA response was smaller than that induced in the adult brain. This may explain why in mature brain the LTD is not inducible. As with LFS, the calcium influx through NMDA receptors in infancy may be moderate, but it would be higher in the adults. The reason why adult and infant brain have different NMDA responses remains unknown. It may be due to the different subtypes of NMDA receptors, may be due to different extent of presynaptic neurotransmitter release with same degree of presynaptic action potential, or may be due to more abundant synaptic connection, so that more synapses were recruited with the same intensity of stimulation in the adult brain.

成果自評：

1. We successfully showed that LTD response is age dependent.
2. We showed that LTP response is age dependent.
3. We clearly showed that the NMDA response is also age dependent.
4. Due to the limitation of facilities, we are still not able to clarify whether the age-dependent change of NMDA response was due to the subtype change of NMDA receptors or due to different degree of neurotransmitter release.
5. The electrophysiology laboratory of Department of Pediatrics, National Taiwan University was set up this year. Due to the delay of merchandizer, we completed the setup only after March, 2000. We had very limited time, and we regret that we can not have time to move on to study the NMDA response in juvenile rats.

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