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学 位 論 文 題 目	Analysis of local circuit of the superior colliculus using multielectrode stimulating/ recording system
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論文内容の要旨

The superior colliculus (SC), converging center between the sensory input and motor command, plays a crucial role in controlling of goal directed orientation responses towards novel sensory stimuli. The SC is a laminated structure divided on functional ground into two major divisions, superficial layers (sSC) and deeper layers (dSC). While the sSC receive the exclusively visual information from the retina and visual cortex, the dSC receive visual and non-visual information from a variety of brain regions and send motor command to the gaze control center in the brainstem. In this work, the neuronal connectivity and signal processing in the synaptic level of the SC local circuit has been investigated in the slice preparation obtained from mouse brain. She has introduced the new experimental stimulating/recording system that works with a large population of neurons to solve the problems of the sparse samplings from a few cells of whole cell patch-clamp or one direction of field potential recording in previous studies. This system records field potential simultaneously from 64 locations, 8x8 arrays with 150 μm inter-electrode spaces. Moreover, while recording the field potentials in slice, it is possible to perform whole-cell patch-clamp recording simultaneously.

The interlaminar and intralaminar circuits of the SC are described in this paper. In normal controls, the field responses, shown in negative potential, were restricted to the electrodes adjacent to the stimulation, and no clear responses were observed in the dSC. However, when the GABAergic receptor antagonist, 10 μM bicculline (Bic), was applied, a large, long lasting negative response was evoked in the dSC that followed a positive potential. They demonstrated that the activation zone of such stimulation spreads more than 1 mm in the horizontal direction and its center gradually shifts from the dorsal to ventral portion of the dSC. Then, to clarify the relationship between the field responses and intracellular potentials, she analyzed the electrically evoked responses in individual neurons from sSC and from dSC by whole-cell patch-clamp recording simultaneously with the field potential recordings. After application of Bic, SGS stimulation induced large negative responses in the sSC, which reflected the EPSPs in the neurons. The spread of activity from the stimulation in the superficial layers in the presence of Bic, indicates the existence of visuomotor pathway.

In the second part of this work, the spontaneous burst was exhibited in both field and whole cell recording system when the extracellular solution containing low concentration of Magnesium (Mg^{2+}) and 10 μM Bic was applied. These activities are proposed to share the common mechanism in presaccadic burst activity in SGI neuron. The spontaneous activities in all 64-channel exhibited the similar pattern to the burst that evoked by electrical stimulation, beginning with the short duration negative filed responses which appeared in the sSC, whereas the positive responses in the dSC. Then, the long and large negative potential happened in the dSC. Various characteristics of spontaneous bursts were recorded, from single burst to complex burst. However, the pattern that burst propagated from the sSC to the dSC is maintained in all spontaneous activity of all tested slices. In addition, she has investigated the responses elicited by the photostimulation system in the various depth of the SC coronal slice. Surprisingly, the SGI stimulation exhibited the less spread of activity than SGS or SO stimulation. This suggests that the amplified network in the sSC is necessary for the wide spread of activity in the dSC. This finding supports that the burst activity propagation is initiated in the sSC.

In the third part, lateral connection in the sSC, the horizontal slice comprising SGS and SO layer was tested. The horizontal slice comprised *stratum griseum superficial* (SGS) and *stratum opticum* (SO) layers were placed on the array of microelectrodes attached the SO layer toward the planar electrodes. The excitatory postsynaptic potential or inhibitory postsynaptic potential in the SGS neurons, elicited by stimulations from one of the planar electrodes to the SO layer, were recorded by whole cell patch clamp recording. She analyzed the rostrocaudal and lateromedial direction of evoked responses that depend on the distances between the stimuli and the neurons in two stimulus protocols, single pulses and multiple pulses (200Hz, 50 ms). The center-surround competitive interaction was found clearly in the high frequency repetitive stimuli condition. The excitatory responses were prominent when stimulations were initiated at the closest electrodes. It is indicated that those short excitatory connections are surrounded by the long inhibitory projection. The neural network mediating the nearby excitatory and distant inhibitory connections was investigated in the voltage clamp recording. The multiple stimuli, 200 Hz, were tested in SGS neurons with two different holding potentials, at -80 mV and 0 mV, to examine the excitatory and inhibitory response separately. The color images demonstrating the temporal profile of responses on the stimulating electrode grid indicate that the both excitatory and inhibitory networks have both short- and long-range connections and they are overlapped. The nearby excitatory and distant inhibitory responses that we have seen in the current clamp recording are the subtracted results between those connections. The different temporal patterns of those connections also mediate their competitive relationship. The excitation rises fast in the first 10 ms, and then decline with faster decay time than inhibition. Therefore, they hypothesize that the distant inhibitory response is contributed by the slower desensitization of the inhibitory than excitatory synaptic connections.

Working with these two recording systems, multielectrode system and patch-clamp recording system, allow them to investigate the neural network of large populations of neuron, while precisely study the intracellular postsynaptic potentials which is important to clarify the functional connectivity in the neural network.

論文の審査結果の要旨

中脳背側にある上丘は、様々な感覚刺激に対して眼球や頭部を向ける指向運動の中枢として、また最近では空間的注意を制御する中枢として多くの研究の対象とされてきた。上丘には層構造があり、浅層は視覚入力を受けるのに対して、中間・深層は視覚以外の感覚入力や大脳皮質からの信号を受けるとともに、脳幹・脊髄に運動指令を出力する。上丘の浅層・深層が脳の他の部位とどのように接続しているかについては多くの研究がなされてきたが、上丘内部の局所神経回路に関する研究はこれまであまり進められてこなかった。本研究において申請者は、マウス上丘スライス標本をデッシュの上に等間隔で配置された64チャンネルの電極の上に置き、フィールド電位記録および刺激法とホールセルクランプ法を組み合わせて、上丘内の広い範囲の多くの神経細胞集団においてどのように信号が伝搬するか、また上丘の異なる部位同士がどのように相互作用するかを解析した。

冠状断切片において、上丘浅層を電気刺激すると通常のリンゲル液中では浅層の最表層部に限局して陰性電位が記録されるのみであったが、細胞外液中に10μMのビクク リンを投与して$GABA_A$受容体をブロックすると、同じ強度の刺激によって短い潜時で浅層には大きな陰性電位、同時に中間層では大きな陽性電位が生じ、その後浅層で生じた陰性電位は次第に中間層から深層に向けて移動していった。このときに短潜時で中間層に誘発される陽性波は浅層で起きている電流シンクに対する電流源であり、平均21ms後から次第に減衰し、その後陰性波に転じて数百ms間持続した。これらの浅層・深層の大きな陰性波はNMDA受容体の拮抗薬であるAPVの投与によって消失したので、NMDA型グルタミン酸受容体依存性であると考えられる。さらに浅層や中間層のフィールド電極記録部位近くのニューロンからホールセル記録を行い、細胞内電位とフィールド電位を記録したところ、浅層の陰性電位はほぼ細胞内電位の興奮性シナプス後電位(EPSP)と時間経過が一致していた。一方で、中間層における陽性電位の期間中にはすでにEPSPが開始していること、細胞ごとでEPSPの持続期間は大きく異なり、必ずしもフィールド電位で記録される陰性波の時間経過とは一致しないことが明らかになった。つまり陰性波は様々な時間経過のEPSPを受けている細胞の集合であることが明らかになった。また、空間的に陰性波は浅層の深部から水平方向に拡大し、中間層で横幅が500μmを超えるように広範囲のニューロン群が同時に脱分極するようになることが見出された。以上の結果から浅層から深層への信号の伝搬機構、特に非常に広範囲のニューロン群が同時に興奮すること、また短潜時で浅層でEPSPが誘発されると中間・深層が電流源になるというフィールド電位の特徴的な生成機構が明らかになった。

他方、浅層内の水平方向の結合関係を明らかにするため、浅層の水平断切片を作成し、視神経層から浅層に入力する線維を64チャンネル電極アレイを用いて電気刺激し、1個の浅層ニューロンからホールセル記録してその効果を確認したところ、刺激に近いところではEPSP、離れたところでは抑制性シナプス後電位(IPSP)が記録された。特に連発刺激によってIPSPがより大きくなる傾向が見られた。このことから上丘の浅層の空間マップで「近傍―興奮、周辺―抑制」というMexican-hat型の水平性の相互作用が内臓されていることが明らかになった。

また電位固定下で電位を抑制性シナプス電流(IPSC)の平衡電位である-80mVに固定して興奮性シナプス後電流(EPSC)を分離して解析し、またEPSCの平衡電位である0mV付近に固定してIPSCを分離して観察したところ、EPSCとIPSCは重複した範囲から誘発されるが、IPSCの誘発範囲の方がやや広く、また持続時間も長いことが明らかになった。このような特徴が上記の「近傍―興奮、周辺―抑制」の神経機構の基盤となっていることが明らかになった。上記の研究はいずれも中脳上丘の局所神経回路の構造と機能の基本的に重要な知見を与えるにとどまらず、動物の行動や注意の生成機構を解明するシステム神経科学の今後の発展に貢献する重要な研究として評価できる。