"The Effects of Wing Bud Extirpation on the Development of the Central Nervous System in Chick Embryos" (1934), by Viktor Hamburger [1]

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German embryologist Viktor Hamburger [4] came to the US in 1932 with a fellowship provided by the Rockefeller Foundation [5]. Hamburger started his research in Frank Rattray Lillie’s laboratory at the University of Chicago [6]. His two-year work on the development of the central nervous system [7] (CNS) in chick [8] embryos was crystallized in his 1934 paper, “The Effects of Wing Bud Extirpation on the Development of the Central Nervous System in Chick Embryos,” published in The Journal of Experimental Zoology [9]. Hamburger was able to use the microsurgical techniques that he had learned from Hans Spemann [10] to show how wing buds influence the development of the CNS in chick [8] embryos. This paper is one of several among Hamburger’s important studies on chick [8] embryos and represents the empirical and theoretical cornerstone for his further research on central-peripheral relations in the development of the nervous system.

“The Effects of Wing Bud Extirpations” begins with an introduction that raises research questions and explains the context of Hamburger’s study. The questions are two-fold. First, what are the effects that peripheral structures, or as Hamburger calls them the peripheral fields, have on the development of the CNS? Second, what are the mechanisms behind such effects? Hamburger acknowledges that Lillie had suggested these questions after conflicting results were obtained in the latter’s laboratory. Hamburger explains that Lillie’s former graduate student, Elizabeth Shorey, had shown in 1909 that electrocauterization of chick [8] wing buds resulted in reduced cell numbers (hypoplasia [11]) in both the spinal ganglia and the anterior and posterior horns of the spinal cord. In 1919, however, Samuel Randall Detwiler [12] at Yale University [13] observed that in the salamander [14] Ambystoma (later called Ambystoma [15]), the lack of forelimb buds only negatively influenced the spinal ganglia and not the spinal cord. Lillie suspected that the trauma caused by electrocauterization might have produced some artifacts in Shorey’s experiments, and believed that this discrepancy necessitated repeating her experiments with other techniques. Hamburger set out to examine Shorey’s experiments using his microsurgical extirpation method, which was less invasive.

In the section of the paper titled “Material Operation,” Hamburger describes the methods by which he removed the wing buds and observed the results. Most of the extirpations were done on 68–72-hour chick [8] embryos. Hamburger began by cutting a small rectangular window in the shell to expose the embryo. After removing the shell membrane under the window, he used a micro glass needle to make a small hole in the amnion [16] that covered the right wing bud. He then further pushed the glass needle to cut through the base of the bud. After lifting the severed wing bud out of the shell, he sealed the opened window on the shell with a thin layer of warm paraffin. Most of the embryos were allowed to develop for four to six days post extirpation. Hamburger then fixed the embryos in Bouin’s fluid, a solution commonly used for fixation and preservation of biological material, and stained them with a blue dye called Heidenhain’s iron hematoxylin. The embryos were then sectioned and examined.

Hamburger presents his results by first giving a description of a typical case among extirpated embryos, numbered S.118. He then summarizes his observations on S.118 and eight similarly treated embryos. These results were in agreement with Shorey’s results. Presenting a cross section of case S.118, in which the right wing bud as well as the shoulder muscle was removed, Hamburger demonstrates that the right half of the spinal cord is significantly smaller than the left. He counts the motor neurons in a series of sections of the anterior horn of the spinal cord, finding that the right anterior horn has 61% fewer neurons than does the left. The posterior horns are less significantly affected—there is only a 22% reduction [17] in the number of neurons on the extirpated side—while the median part of the spinal cord shows no reduction [17] in volume or in number of neurons. In addition, the spinal ganglia are shown to have shrunken in size.

Hamburger analyzes the common patterns and variations manifested in the nine cases he had experimented on. Hypoplasia was observed in the anterior and posterior horns of the spinal cords of all nine extirpated chick [8] embryos, as well as in their spinal ganglia. However, since it was difficult to remove the entire wing structure in every extirpation operation, various portions of muscle structure were left in the embryos. Hamburger notes that the remaining motor neurons in the anterior horns were proportional to the amount of muscle structure left intact. The degree of hypoplasia [11] in the spinal ganglia did not, however, vary in proportion to the changing amount of remaining muscle structures. In these nine cases, cell counts in the anterior horns were reduced by 22–60%, and in the spinal ganglia by around 55%. Hamburger also notes that the brachial plexus in those cases was
normally formed, although the number and the paths of the nerves originating from the plexus were abnormal and irregular on the extirpated side.

The next section of the paper is devoted to a discussion of the results. Hamburger begins by analyzing hypotheses that had been offered to explain size reduction [17] in the CNS following limb-bud removal. Others had proposed that either the high physiological activity of the growing buds, or a kind of neuronal signaling such as the reflex arc generated by the limbs, stimulated the overall growth of the CNS. Hamburger instead maintains that his results support an alternative hypothesis: that every structure within the growing limbs sends targeted stimuli to specifically control its own nervous centers. For instance, muscles send signals that influence the development of the lateral motor centers in the anterior horns, while those from the sensory organs affect the development of the ganglia. Evidence supporting Hamburger’s hypothesis about the specificity of peripheral control concerned the proportional relation between the hypoplasia [11] in the anterior horn and the amount of muscle structure removed. Hamburger also notes how the hypoplasia [11] in spinal ganglia did not vary much between cases because the ganglia reacted to the remaining sensory organs of the skin. Although considerable amounts of skin were removed in all cases, there was little variation in the amount removed. This, Hamburger reasons, explains why variation in the hypoplasia [11] displayed in the spinal ganglia was limited.

Hamburger further reflects on what cell processes and through what mechanisms the peripheral stimuli regulate the development of the CNS. Since cells confined within the spinal cord first undergo mitosis [18], and then start differentiation [19] and migration, Hamburger hypothesizes that the stimuli from the peripheral structures might affect any of the three steps involved in the formation of neurons: proliferation, differentiation [18], and migration. Regarding how the stimuli are transferred from the peripheral structures to the central neurons, Hamburger suggests that the nerve fibers serve to transport the peripheral stimuli centripetally to the undifferentiated (also called uncommitted) neurons in the central portions of the developing CNS. With these hypotheses Hamburger suggests that the peripheral stimuli induce undifferentiated cells to join the CNS through division, movement, or differentiation [19]. Many saw his hypothesis as an articulation of the recruitment hypothesis in the language of induction [20].

Hamburger also proposes that the intrinsic growth of central neurons and the stimuli from peripheral structures both contribute to the development of the CNS, calling this process the synergetic principle for the development of the spinal cord. He notes that even when all the limb muscle structures were removed, there was still 40% motor neuron [21] development in the anterior horn. This independent partial growth, says Hamburger, shows that there was constituent growth in motor columns that was intrinsically determined without the intervention of the peripheral influences. He thus concludes that the intrinsic central growth and the influences from the peripheral structures coordinate to determine the final shape of the CNS in the chick [8].

In the section “Discussion of the Results Obtained in other Experiments,” Hamburger compares, analyzes, and synthesizes others’ results regarding central-peripheral relations in birds [26], mammals, and amphibians [23]. Reviewing relevant literature, he reports that research into abrachia, a congenital condition in which the arms are missing, suggests that the congenital lack of limbs in mammals correlates with diminished size of the CNS, with patterns similar to that observed in birds [22]. He concludes that central-peripheral relations in mammals and humans [24] resemble those demonstrated in chicks. Some amphibians [23], however, seem to be quite different in how their peripheral influences affect the development of the CNS following limb-bud removal. Others had proposed that either the high physiological activity of the growing buds, or a kind of neuronal signaling such as the reflex arc generated by the limbs, stimulated the overall growth of the CNS. Hamburger instead maintains that his results support an alternative hypothesis: that every structure within the growing limbs sends targeted stimuli to specifically control its own nervous centers. For instance, muscles send signals that influence the development of the lateral motor centers in the anterior horns, while those from the sensory organs affect the development of the ganglia. Evidence supporting Hamburger’s hypothesis about the specificity of peripheral control concerned the proportional relation between the hypoplasia [11] in the anterior horn and the amount of muscle structure removed. Hamburger also notes how the hypoplasia [11] in spinal ganglia did not vary much between cases because the ganglia reacted to the remaining sensory organs of the skin. Although considerable amounts of skin were removed in all cases, there was little variation in the amount removed. This, Hamburger reasons, explains why variation in the hypoplasia [11] displayed in the spinal ganglia was limited.

The article ends with Hamburger reiterating that his results are in full agreement with those described by Shorey and that the discrepancy of results obtained from chick [8] and salamander [14] embryos could be explained by the genuine structural differences in their central nervous systems. Based on careful experiment, observation, and analysis, “The Effects of Wing Bud Extirpation” nevertheless was blemished by several theoretical assumptions that were later proved invalid. It turns out that the peripheral stimulus does not regulate neuron [21] proliferation, differentiation [19], or migration, as the recruitment hypothesis suggests, but instead depends on cell deletion processes. In 1942 the Italian embryologist Rita Levi-Montalcini [25] showed that central neurons were developed through overproduction followed by selective deletion, and that it was at the cell deletion stage that the peripheral stimuli intervened. Beginning in 1947, Hamburger and Levi-Montalcini joined forces to reinvestigate the issue. Their efforts led to the revision of Hamburger’s theory, which they discuss in their co-authored paper “Proliferation, Differentiation and Degeneration in the Spinal Ganglia of the Chick Embryo under Normal and Experimental Conditions,” published in 1951.

Sources
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