point, two tetramers were joined together by a disulfide linkage and therefore fractionated from the bulk of the tetramer on the Sephadex column.

Thus, the minor H2A variants substitute for a fraction of the major H2A variants in the H2A:H2B dimer. This point is particularly relevant for H2A.Z because its sequence diverges considerably from those of the major H2A variants and because it differs from them in its pattern of modification. In addition, the finding that ubiquitination seems to have no effect on histone association is substantiated and is extended to include the minor H2A's and H2B.

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Fetal Brain Transplants: Reduction of Cognitive Deficits in Rats with Frontal Cortex Lesions

Abstract. *Frontal cortex and cerebellar tissue from fetal rats was implanted into the damaged frontal cortex of adults. Cognitive deficits in spatial alternation learning that follow bilateral destruction of medial frontal cortex were reduced in rats with frontal cortex implants but not in those with implants of cerebellum. Histological evaluation showed that connections were made between the frontal cortex implants and host brain tissue.*

Interest in the problem of recovery from brain injury is growing, and a number of new approaches are being tried (*1*). One of the more novel and interesting of these involves the transplantation of embryonic brain tissue directly into the damaged brain of a mature recipient (*2*). In recent experiments the behavioral deficits associated with damage to the nigrostriatal or fimbria-fornix systems have been diminished by implanting fetal dopaminergic neurons or solid embryonic septal grafts, respectively, into the lesion sites (*3, 4*). Anatomical studies with anterograde and retrograde tracers have also shown that the transplants can establish connections with the host brain (*5*), while electrophysiological experiments show that the neural implants are capable of forming functional synapses (*6*).

Despite these achievements, the ability of brain grafts to mediate behavioral recovery after bilateral cortical ablations has still not been systematically investigated. We report here that the impairments in cognitive functioning caused by damage to the medial frontal cortex are significantly reduced by the implantation of fetal frontal cortex into the lesion site. Furthermore, injections of the enzyme horseradish peroxidase (HRP) show that the transplants and the host brains establish afferent neuronal connections.

Twenty-nine male Sprague-Dawley rats (Charles River; CD) approximately 105 days old at the time of surgery were used. Eight animals served as unoperated controls with sham incisions. The medial frontal cortex of the remaining 21 animals was damaged bilaterally by aspiration (*7*). Seven days after surgery 14 animals were implanted with fetal frontal cortex ($N = 8$) or fetal cerebellar tissue ($N = 6$) (*8*). (The unoperated controls and the seven lesion animals not receiving implants were anesthetized at this point and their wounds were reopened.) The transplanted neural tissue was obtained from CD rat fetuses on day 21 or 22 of gestation and placed into the cavity created by the removal of the medial frontal cortex (*9*). The implants had a volume of approximately 6 mm^3 and were placed bilaterally directly into the area of damage.

On the fourth day after transplantation all 29 animals began training on a spatial alternation task in a T-maze (*10*). Spatial alternation requires the water-deprived rat to enter the goal arm opposite the one entered on the previous trial in order to receive a 0.15-ml water reward. This test has been used to determine the effects of frontal cortex damage (*11*). Ten trials per day constituted a testing session, and animals were tested 5 days per week. When an animal made 19 of 20 choices correctly during two consecutive test sessions, or when 30 test sessions had been completed, testing was terminated for that rat. After behavioral testing, and between 78 and 155 days after transplantation, the rats that had received frontal cortex or cerebellar tissue were given injections of the retrograde transport marker HRP in the transplant or the host brain to determine whether afferent connections had been established between these neural regions (*12*).

We found that transplants of frontal cortex, but not cerebellar tissue, facilitated recovery from the lesions (Fig. 1). An analysis of variance revealed significant differences among the four groups in the number of days needed to meet our most stringent criterion, the making of 19 of 20 choices correctly in two consecutive days [$F(3, 25) = 10.91$, $P < 0.01$]. Randomization tests for two independent groups revealed that rats receiving frontal cortex performed significantly better than the lesion group that did not receive brain transplants in terms of number of days needed to make nine of ten choices correctly in 1 day ($P < 0.01$), number of days needed to make 18 of 20 choices correctly in two consecutive days ($P < 0.05$), total number of errors divided by number of trials needed to meet the most stringent criterion ($P < 0.05$), and number of perseverative errors divided by number of trials needed to meet the most stringent criterion ($P < 0.05$).

Animals that received frontal cortex scored significantly better than the group given cerebellar tissue on days needed to make nine of ten choices correctly in 1 day ($P < 0.05$) and number of perseverative errors divided by number of trials needed to make 18 of 20 and 19 of 20 choices correctly over 2-day periods

($P < 0.05$). Four of the six animals that received cerebellar transplants and three of the seven animals with cortical injuries and no transplants never met our most stringent criterion. In contrast, only one of the eight lesion animals receiving frontal cortex tissue failed to reach this criterion.

The unoperated control animals scored significantly better than the three lesion groups on all of the measures we employed. No significant differences were observed between the cerebellar transplant group and the group with lesions only.

After the behavioral testing, five animals with frontal cortex transplants and three with cerebellar transplants were used for an anatomical evaluation of afferent connections. In the exposed brains the grafts were clearly visible only in those animals that had been implanted with frontal cortex tissue. These grafts were located in the rostral portion of the lesion cavity and appeared to the naked eye as oval, whitish lumps. HRP was then injected ipsilaterally into the graft ($N = 3$) or the host cortex ($N = 2$). In cerebellar transplant rats we made unilateral injections of HRP into the cortex ($N = 3$) since no transplanted tissue was visible. Rejection of the cerebellar transplants may have been caused by a difference in specific growth factors between the frontal cortex of the host and the cerebellar tissue (*13*). The HRP-injected brains were processed by the highly sensitive tetramethylbenzidine procedure and the remaining brains were prepared for histological analysis by staining for Nissl substance (*12*).

Histological examination revealed that transplants either formed continuous bridges connecting the injured hemispheres or formed separate grafts, each adhering to the host cortex. At the points of attachment between graft and host there were areas of continuity, some of which exhibited glial scarring (Fig. 2, A to C). Light microscopic evaluation of the cresyl violet–stained sections revealed little internal order in the transplants and no laminar arrangement of neurons characteristic of the frontal cortex. The perikarya in the grafts varied in size and occasional large neurons were seen (Fig. 2C). In all brains with lesions, bilateral damage included the medial frontal portion of the cortex from the tip of the frontal pole to at least the genu of the corpus callosum, and in several cases there was some minor involvement to the head of the caudate nucleus.

In all three brains with HRP injections into the frontal grafts the HRP was restricted to one side of the transplant. In one animal the HRP reaction was confined to the transplant, while in the other two there was some minor involvement of the adjacent cortex. In each case, however, labeled neurons were observed in the adjacent host cortex as well as in the medial dorsal and anterior thalamic nuclei (Fig. 2D). Areas of host brain found to project to frontal transplants were areas known to have efferent connections with portions of normal frontal cortex (*7*). In addition, retrogradely labeled cells were found in the contralateral portion of the transplant, suggesting that intratransplant connections had been established.

In each brain with a frontal cortex transplant and an HRP injection into the host cortex, labeled perikarya were ob-

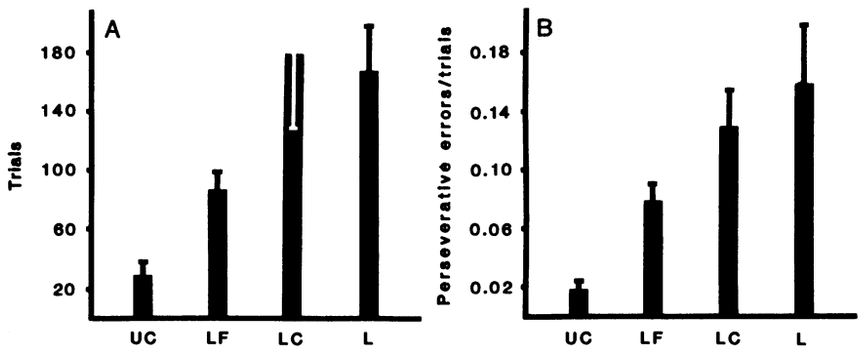

Fig. 1. (A) Mean number of trials needed to make nine of ten choices correctly during one test session. (B) Mean number of perseverative errors divided by number of trials needed to meet the most stringent criterion. Abbreviations: *UC*, unoperated controls; *LF*, frontal cortex lesions with grafts of fetal frontal cortex; *LC*, frontal cortex lesions with grafts of fetal cerebellar tissue; and *L*, frontal cortex lesions without implants of fetal tissue.

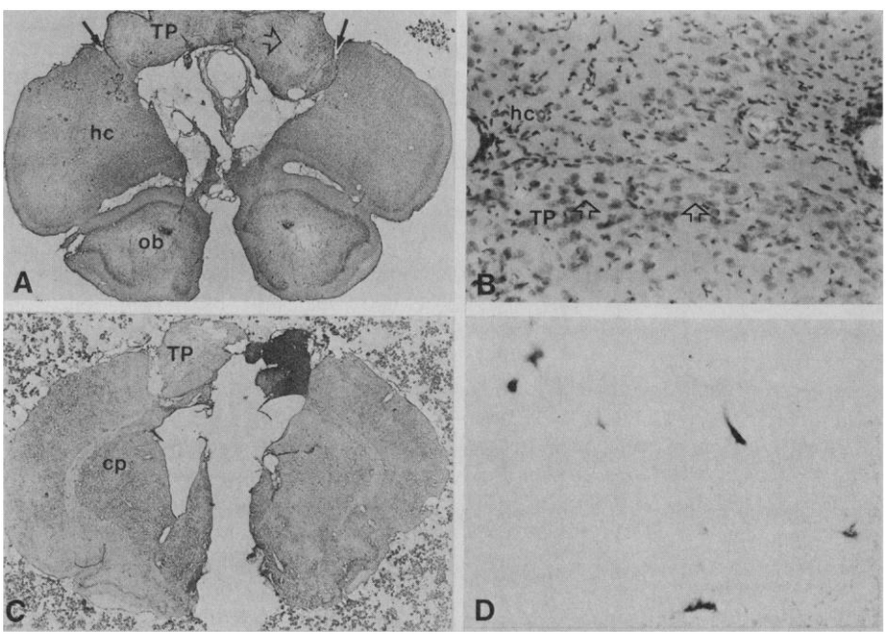

Fig. 2. (A) Transverse section of rat brain stained for Nissl substance, showing the position of a fetal cortex transplant (*TP*) in the anterior region of the frontal pole 78 days after transplantation. The transplant has bridged the cavity produced by aspiration. Neuron-free patches appear in the transplant (open arrow). Filled arrows indicate the points of attachment between the host tissue and the transplant. Abbreviation: *ob*, olfactory bulb (×10). (B) Transplant tissue stained for Nissl substance, showing the area of attachment between host tissue and implant in another animal. The host cortex (*hc*) is situated in the upper portion of the micrograph and the transplant in the lower portion. Note the large neurons located at the host-transplant border (open arrows) (×50). (C) Coronal brain section counterstained with cresyl violet, showing a cortical transplant unilaterally injected with HRP. The injection site appears black and there is virtually no spread of injectate into the adjacent cortex. At this level the transplant appears as two separate islands of tissue; however, at more anterior levels these islands are joined. Abbreviation: *cp*, caudate-putamen (×10). (D) Uncounterstained section showing retrogradely labeled anterior thalamic neurons in the host after the injection of HRP into the cortex transplant (×100).

served in the transplant. However, there was a slight diffusion of the HRP from the host cortex into the transplant.

Functional recovery from brain damage in animals with transplants of neural tissue may be due to factors other than connectivity between the transplant and host brain. It is possible that fetal brain grafts release neuronotrophic substances, such as polyamines and specific nerve growth factors. These may promote functional recovery by altering glial activity or neurotransmitter levels or by changing membrane receptor properties in the tissue surrounding the graft. Although the specific mechanisms remain to be discovered, these findings indicate that transplants of cortical tissue in adult rats are capable of enhancing behavioral recovery after bilateral brain injury.

Dunnett *et al.* (*4*) found that implants of fetal septal tissue promoted recovery of a learned discrimination in rats with damage to the fimbria-fornix system. These animals were able to solve a rewarded, spatial alternation task significantly faster than rats with similar damage but without transplants. However, animals with grafts did not perform as well as intact control animals on the spatial alternation task. These findings are similar to our own, despite the fact that Dunnett *et al.* waited 7 months before beginning behavioral training whereas we began testing just 4 days after transplantation. It is reasonable to conclude that the transplanted tissue begins to mediate behavioral recovery soon after transplantation and remains functional for almost a year, and perhaps for the rest of the animal's life.

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Effects of Serotonin on Memory Impairments Produced by Ethanol

Abstract. *Subjects treated with low or high doses of ethanol demonstrated impaired memory, particularly in tests involving the recall of poorly learned information. Zimelidine, an inhibitor of serotonin reuptake, reversed this ethanol-induced impairment. The serotonin neurotransmitter system may mediate learning and memory in humans and may determine some of the effects of alcohol on higher mental functions.*

While ethanol has been shown to disrupt many higher mental functions, its effects on learning and memory processes have been studied the most extensively. This is due in part to the precision with which these aspects of cognition can be measured. However, we still know little about the behavioral and biological mechanisms underlying ethanol-related changes in learning and memory (*1–3*). At intoxicating doses, ethanol alters many information-processing components, including quantitative and qualitative aspects of how events are encoded, the shift of information from short- to long-term memory, retrieval processes, and memory consolidation (*1–7*). A complete understanding of these complex cognitive changes depends on our knowledge of the psychobiology of memory and learning, which remains incomplete (*8–10*). A better understanding of how ethanol alters information processing would also be useful for elucidating the biology of information processing and might generate new tools for treating ethanol-related cognitive impairments.

This study was designed to ascertain whether zimelidine, a relatively specific blocker of serotonin reuptake (*11*), can attenuate the impairing effects of ethanol on memory and learning. We found that poorly learned but not well-learned information was subject to ethanol-induced memory impairment in a dose-dependent fashion and that treatment with zimelidine prevented this cognitive disruption.

In a preliminary experiment we found that a single dose of zimelidine reversed ethanol-induced impairment of memory, as measured by a battery of standardized procedures that test vigilance, learning, and memory functions (*12*). This prompted us to conduct the present experiment. The subjects were ten male volunteers, all with at least some college education. They were 22 to 27 years old, free of medical or psychiatric illness, within 15 percent of their ideal body weight, and had normal liver enzyme values. Their first-degree relatives were free of alcoholism. The subjects were informed about the nature of the study and gave their signed consent to participate as volunteers.

Study design consisted of a 10-day hospitalization, preceded or (in half the subjects) followed at least 2 weeks later by three successive outpatient days. After receiving an initial 200-mg dose of zimelidine on the morning of admission, the subjects were given 100-mg doses twice a day (morning and evening) throughout their hospital stay. Each subject served as his own control under six different test conditions. On each test day they had a light breakfast between 0700 and 0800. On arrival at the laboratory between 0900 and 1000, the subjects were given breath alcohol analyzer tests to ensure that their systems were ethanol-free. Between 1000 and 1100 each subject ingested two tablets containing a placebo or 100 mg of zimelidine. (Zimelidine would be expected to produce its