and attack, and so the production of analgesia during such times would be adaptive. The same considerations would suggest that the analgesia should be terminated when the situation is safe and danger no longer present, and this is what we have found. This would allow the return of recuperative behaviors. It would seem that pain is a finely tuned sensory system, with an interplay of pain-inhibitory and antipain-inhibitory processes determining the intensity of the sensation.

Antianalgesia mechanisms may be important for the development of opiate tolerance and perhaps dependence, because they involve opioid and antiopioid endogenous substances. The spinal application of proglumide and other CCK antagonists reverses and blocks the development of morphine tolerance (*3*). The implications are that the activation of opiate receptors by morphine triggers CCK release and that each application of morphine with consequent opiate-receptor activation enhances either the output of CCK or the sensitivity of CCK receptors. Thus tolerance develops because the opponent CCK system strengthens with repeated administrations of morphine. In our experiments L-365,260 blocked the effect of the safety signal, which itself interfered with morphine's analgesic action. This suggests that the development of tolerance to morphine might differ, depending on whether morphine is administered in a relatively safe or in a dangerous and fear-arousing environment. Tolerance and perhaps dependence ought to develop more rapidly in a safe environment, where CCK systems are already augmented. Indeed, morphine dependence does not readily develop when morphine is delivered in hospitals, a relatively fear-provoking environment (*17*). Moreover, morphine reactivity is enhanced by mildly fearful and arousing circumstances (*18*). Thus, some of the individual differences in the development of opiate tolerance and dependence might be traceable to the stressfulness of the situation in which the morphine is received, and the interplay between endogenous analgesia and antianalgesia mechanisms may be the mediating link.

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Changes in the Sensory Processing of Olfactory Signals Induced by Birth in Sheep

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After giving birth, sheep and many other species form a selective bond with their offspring based on the sense of smell. Processing of olfactory signals is altered to allow the animals to perform this selective recognition. Lamb odors have little effect on either neurotransmitter release or electrical activity of neurons in the olfactory bulb before birth. However, after birth there is an increase in the number of mitral cells, the principal cells of the olfactory bulb, that respond to lamb odors, which is associated with increased cholinergic and noradrenergic neurotransmitter release. Selective recognition of lambs is accompanied by increased activity of a subset of mitral cells and release of glutamate and γ-aminobutyric acid (GABA) from the dendrodendritic synapses between the mitral and granule cells. The relation between the release of each transmitter after birth also suggests an increased efficacy of glutamate-evoked GABA release.

In sheep, an enduring bond between a mother and her lambs is established very rapidly, usually within 3 hours of parturition (*1*). Before giving birth, pregnant ewes find the smell of amniotic fluid repulsive and are indifferent toward, or violently reject, approaches by lambs. Immediately after the fetal membranes rupture, however, the ewe is attracted to the smell of amniotic fluid (*2*) and, within a few minutes of birth, starts to lick and sniff her newborn lamb and encourages it to adopt a standing posture and suckle. The ewe's interest in lambs and her ability to selec-

tively recognize her own offspring depend on the effect of parturition to trigger her interest in their sensory cues, which are primarily olfactory (3). During parturition, signals from the stimulation of the vagina and cervix feed back to the brain to induce the ewe both to become maternally responsive toward lambs and to form a selective bond with them (4). Profound neurochemical changes occur in the olfactory bulb of the sheep as a result of parturition or artificial stimulation of the vagina and cervix (5). We therefore investigated how the organization of olfactory processing in the brain is altered electrophysiologically and neurochemically to accommodate the animal's behavioral requirement of recognizing its own lambs.

Electrophysiological recordings were made from olfactory bulb neurons in the same four conscious Dalesbred ewes before and after they gave birth. During recordings, the ewes were comfortably suspended in a canvas hammock (6). Single-cell activity was recorded extracellularly with glass-coated tungsten microelectrodes introduced into the olfactory bulbs with a hydraulic microdrive by means of 18-gauge stainless steel guide tubes surgically implanted directly above the lobes (7).

A total of 188 cells responded differentially to the various chemical and biological odors presented (105 recorded before birth and 83 after birth) (8). Histological examination revealed that these cells were located mainly in the mitral cell layer. In recordings made during the last 2 months of pregnancy, none of these cells responded preferentially to lamb or amniotic fluid odors (Fig. 1A). Indeed, in only 11 cells (10%) were these odors capable of eliciting any significant change in firing rate. The majority of cells (72%) responded preferentially to food odors, and some responded to wool or amyl acetate (Fig. 1A). Between 3 days and 4 weeks after birth, there was a dramatic increase in the proportion of cells in the same area of the olfactory bulb that responded preferentially to lamb odors (60%) ($P < 0.05$). The majority of cells that responded to lamb odors (70%) did not differentiate between the odor of the ewe's own lamb and that of an alien lamb (Fig. 1B) and were remarkably resistant to habituation (9). However, a proportion of the cells (30%) did respond preferentially to the odor of the ewe's own lamb (Fig. 1C), and in all cases the smell of the lamb's wool was almost as effective a stimulus as that of the whole lamb. A small proportion of cells was also found that responded preferentially to amniotic fluid odors (11%) (Fig. 1A). There was a large reduction in the number of cells that responded primarily to food odors (11%) ($P < 0.05$), whereas the proportion that responded primarily to amyl acetate and adult wool odors remained unchanged. These results indicate that, although the odors of lambs have almost no influence on the activity of olfactory bulb neurons during the period before birth, when lambs have no behavioral attraction, they are the most potent olfactory stimulus of those tested in the period after birth, when the recognition of lamb odors has a very high behavioral priority. Moreover, a proportion of cells responds preferentially to the odor of the lamb with which the ewe has formed a selective bond.

The olfactory bulb is a relatively simple trilaminar structure, and its network comprises three basic neural types (Fig. 2E). The mitral cells, which show this increased responsiveness to lamb odors after birth, receive and transmit olfactory signals, and their activity is modulated at their apical dendrites by periglomerular cells and at their lateral dendrites by granule cells (10). Intrinsic connections within this network contain both excitatory and inhibitory amino acid transmitters and dopamine (11). Transmission among neurons in the network is further influenced by centrifugal projections from noradrenergic (12), cholinergic (13), and serotonergic (14) neurons that lie deep in the brain.

To further understand how the mitral cells increase their responsiveness to lamb odors, we used in vivo microdialysis to measure their effect on the release of acetylcholine (ACh), amino acid, and monoamine transmitters in the olfactory bulb before and after birth. In this part of the study, nine adult Clun Forest ewes were anesthetized 4 to 6 weeks before giving birth (7) and implanted bilaterally over the olfactory bulbs with 18-gauge stainless steel guide tubes placed with x-ray guidance. The guide tubes were inserted through the nasal sinus and directed at right angles to the rostral pole of the bulb. In this way, the microdialysis probes (CMA-10, 4-mm membrane length, CMA/Microdialysis, Stockholm, Sweden) preferentially sampled the external plexiform layer and monitored the neurotransmitter interactions between granule and mitral cells. Neurotransmitter release in response to lamb odors was measured 4 to 24 hours before birth and again in the same ewes 24 hours after birth. Samples were collected at 5-min intervals before, during, and after a 10-min exposure to lamb odors. Neurotransmitter concentrations were measured by high-performance liquid chromatography (15). Behavioral tests were given to all the ewes 6 hours after birth to confirm that they had all formed a selective recognition bond with their lambs.

After parturition, when ewes had established a selective bond with their lambs, the odors of these lambs, but not those of alien ones, increased the release of both the excitatory amino acid glutamate (Fig. 2A) and the inhibitory one, γ-aminobutyric acid (GABA) (Fig. 2B). Release of another intrinsic transmitter, dopamine, was not influenced by lamb odors. These changes in glutamate and GABA release occurred only during the first 5 min of exposure to the lamb odor, and the increase of GABA after

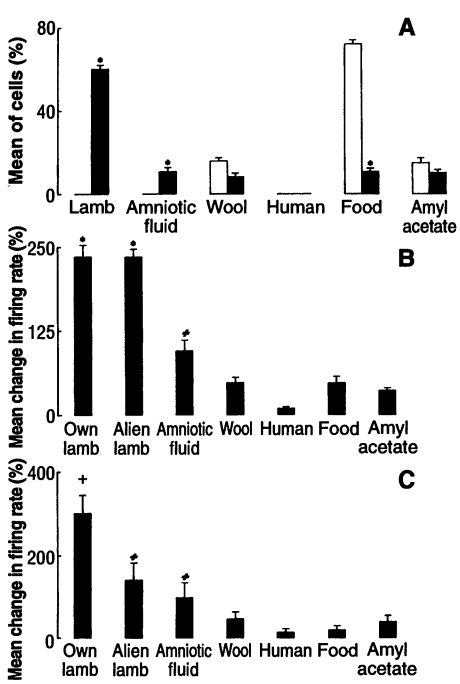

Fig. 1. The effects of birth on the responses of mitral cells to odors. (**A**) Percentage of mitral cells (mean ± SEM) that responded preferentially [with a significantly greater change in response than to other odors (*t* test)] to the different classes of odor used. Open histograms represent data from 105 cells recorded before birth (baseline rate, 5.6 ± 0.5 Hz) and closed histograms from 83 cells after birth (baseline rate, 4.7 ± 0.6 Hz; $n = 4$ animals). Asterisk, $P < 0.05$ compared to values obtained before birth (paired *t* test). (**B**) Change in firing rate of 38 mitral cells recorded after birth that responded preferentially to all lamb odors (baseline rate, 4.5 ± 0.9 Hz). Asterisk, $P < 0.05$ compared to all other odors except alien lambs or a ewe's own lamb; #, $P < 0.05$ compared to wool, human, food, or amyl acetate. (**C**) Change in firing rate of 11 mitral cells recorded after birth that responded preferentially to own lamb odors (baseline rate, 5.0 ± 1.2 Hz). Statistics are as in (B); +, $P < 0.05$ compared to all odors including alien lamb. Open bars, prepartum; dark bars; postpartum.

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birth was significantly greater than that of glutamate (mean ± SEM = 89 ± 33.4% for GABA; 52 ± 25.8% for glutamate; $P < 0.05$, Wilcoxon test). Basal release of GABA and glutamate in the period after birth was also significantly higher than in the period before birth (Fig. 2, A and B), and, again, this change in GABA release was significantly greater than that of glutamate (117.1 ± 20.5% for GABA; 61.2 ± 18.6% for glutamate; $P < 0.05$).

Because the GABA-containing granule cells are intrinsic bulbar neurons excited by mitral cells and provide feedback inhibition to the mitral cells by way of reciprocal dendrodendritic synapses, the proportionately higher release of GABA compared to glutamate might be explained in terms of a changed efficacy of glutamate at these synapses after birth. We further examined this possibility by comparing the regression slopes for the two transmitters in the periods before and after birth. Glutamate and GABA release were correlated both before and after birth in all animals (Fig. 3). A similar relation did not occur with the centrifugal inputs to the bulb because glutamate release was not always correlated with that of ACh, norepinephrine (NE), or serotonin. Although both glutamate and GABA release were positively correlated before and after birth, the regression slopes for the two periods were significantly different (Fig. 3). This overall increase in both glutamate and GABA release in the period after birth is synonymous with more mitral cell activity in response to lamb odors, whereas the significant shift in the regression slope may be a result of an increased efficacy of glutamate in promoting GABA release. Such enhancement of neurotransmission at the granule to mitral cell synapses after birth, and in response to the odor of the animal's own lamb, may not simply produce more inhibition but could result in a change in the firing frequency of those neurons that are coded for the odor of the lamb (*16*). This situation would then produce a bias in the network with respect to these odors.

These findings reveal a change in the processing of biologically relevant odors in the olfactory bulb of the ewe after birth. Changes in bulbar neuronal activity occur as part of a learning process induced by mating in mice (*17*), by stroking of rat pups at birth (*18*), and by aversive conditioning of rabbits (*19*) to odors. Lesions of the noradrenergic projections to the bulbs prevent olfactory learning (*20*) and the ewe's selective recognition of her lambs (*21*). In sheep, the centrifugal projections also provide an increase in NE and ACh release in the bulb at birth (*5*). During the 24-hour period before parturition, presentation of a lamb before a ewe giving birth does not affect NE or ACh release in the ewe's olfactory bulb, although by 24 hours after birth, it stimulates the release of both neurotransmitters (Fig. 2, C and D). These centrifugal projections are essential for olfactory memory formation (*20*) but, because NE and ACh release also occurs in response to lamb odors after memory formation, they may also be required for recall. However, unlike the release of GABA and glutamate, the release of ACh and NE fails to distinguish between a ewe's own and an alien lamb, which suggests that ACh and NE are important for the identification of lamb odors in general rather than for the selective recognition of a ewe's own lamb. Indeed, ewes are interested in any lambs after birth even though they rapidly reject those that are not their own.

The capacity of olfactory bulb circuits to respond to sensory information, contingent upon parturition, in such a way that plastic changes render the processing of olfactory information different on subsequent exposures to the stimuli, is similar to the plasticity that occurs in the hippocampus as revealed by long-term potentiation (LTP) (*22*). Indeed, the olfactory bulbs have much in common with the CA3 region of the hippocampus. Both olfactory mitral and hippocampal pyramidal cells fire in bursts (*23*) and are susceptible to seizure (*24*). Blockage of GABA facilitates both olfactory recognition and LTP in hippocampal CA3 neurons, and these processes are dependent on NE (*25*). Not only has evolution been conservative in its neural mechanisms for learning (the combination of noradrenergic, GABA-containing, and glutamatergic synapses frequently occurs in learning mechanisms) (*26*) but, in this particular case, changes in neural processing of importance for lamb recognition occur at the initial relays in the olfactory system. Learning abilities in animals are adaptive specializations shaped by natural selection to solve specific problems posed by their environment (*27*). For sheep, the elec-

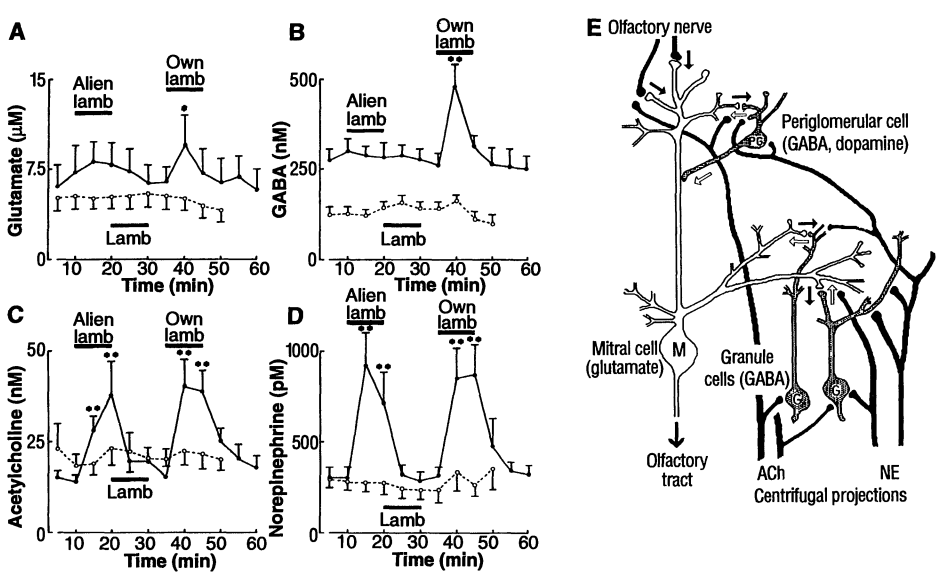

Fig. 2. The effects of birth on neurochemical release in response to lamb odors. Concentrations of glutamate (mean ± SEM) (**A**), GABA (**B**), ACh (**C**), and NE (**D**) in sequential 5-min microdialysis samples taken from the olfactory bulbs of nine sheep during exposure to lamb odors (for 10 min) 4 to 24 hours before birth (dotted line) and 24 hours after birth (solid line). The periods of exposure to lamb odors are indicated by horizontal black bars. Double asterisks, $P < 0.01$; single asterisk, $P < 0.05$ compared to the two control samples immediately before odor stimulation. Results are an overall mean concentration from both olfactory bulbs. A ewe's own lamb and alien lamb odors were presented in a random order. (**E**) Schematic drawing showing the olfactory bulb and the synaptic organization of its neurotransmitters (in parentheses). Solid arrows, excitatory connections; hollow arrows, inhibitory connections.

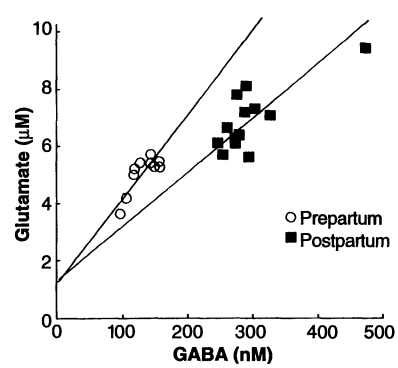

Fig. 3. A regression analysis of mean glutamate and GABA concentrations in microdialysis samples taken from the olfactory bulbs before and after birth. Glutamate and GABA release are significantly correlated before ($r = 0.78$, df = 8, regression coefficient = 25 ± 7, 95% confidence limits = 0.28 to 0.93, $P = 0.008$) and after birth ($r = 0.77$, df = 11, regression coefficient = 49 ± 12, 95% confidence limits = 0.38 to 0.92, $P = 0.002$), but the slopes are significantly different (df = 21, $t = 3.34$, $P = 0.003$).

trophysiological and neurochemical changes that occur in the olfactory bulb after birth represent such an adaptive specialization in response to a behavioral requirement of the selective recognition of their own offspring.

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7. General anesthesia was induced with an intravenous injection of 400 mg of sodium methohexitone and then with an injection of halothane. Full sterile precautions were taken during surgery, and the animals were allowed 3 to 4 weeks to recover.
8. A differential response to odors was defined as a significant neuronal response to some, but not all, of the odor stimuli tested. A total of 107 cells did not fulfill this criterion (60 before birth and 47 after birth). Odor stimuli included three foods (oats, hay, and nuts), amniotic fluid from an alien lamb, a human hand, wool shorn from an unfamiliar adult female sheep, amyl acetate, and lamb's wool, either shorn or on the lamb. After birth, the wool fibers of both alien lambs and a ewe's own lambs were used as stimuli. The stimuli were presented for 10 s on two or three occasions and in random order (for statistics the firing rate during the previous 10 s (20 0.5-s bins) was compared to that during the odor stimulus (20 0.5-s bins). At least 1 min was allowed to elapse between successive odor stimuli.
9. Although firing rate changes in response to odors were based only on the duration of a 10-s stimulus, the responses of the cells to food odors before birth, and to lamb odors after birth, could be maintained for several minutes or more without significant habituation.
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Initiation of Deregulated Growth of Multipotent Progenitor Cells by *bcr-abl* in Vitro

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Expression of the *bcr-abl* oncogene in multipotent progenitor cells (MPPCs) is implicated as a key event in the development of chronic myelogenous leukemia. Bone marrow enriched for MPPCs was infected with a retrovirus that carried *bcr-abl*. The mixed-lineage colonies that resulted were responsive to growth factors and could differentiate. These cells later became growth factor–independent but, when injected into severe combined immunodeficient mice, were not leukemogenic. Thus, the presence of *bcr-abl* alone does not cause growth factor independence, although it initiates a stepwise process. This system may prove useful in the study of other oncogenes that cause leukemia.

The Philadelphia chromosome (Ph^1) is the molecular hallmark of human chronic myelogenous leukemia (CML) (*1*, *2*). A consequence of this interchromosomal translocation is the formation of the chimeric *bcr-abl* oncogene (*3*); the product of this *bcr-abl* gene (p210) is an activated form of the c-*abl* protein tyrosine kinase (*4*). Human CML exhibits a biphasic clinical course that originates with a clonal expansion of a pluripotent or multipotent hematopoietic progenitor cell (MPPC) (*1*). During the disease's initial chronic phase, which may last for several years, committed myeloid progenitor cells that retain their ability to differentiate increase in number. Progression of the disease is marked by accelerated growth of immature myeloid or lymphoid cells that no longer differentiate. The presence of Ph^1 and the $p210^{bcr\text{-}abl}$ protein in cells during both chronic phase and acute blast crisis suggests that *bcr-abl* expression is an important factor in CML pathogenesis (*5*).

It is not known if *bcr-abl* can directly stimulate the growth of MPPCs or if other genetic alterations that precede the formation of Ph^1 are responsible for the expansion of the MPPC clone observed in CML (*6*). In vivo murine studies have shown that *bcr-abl* can induce various hematopoietic malignan-

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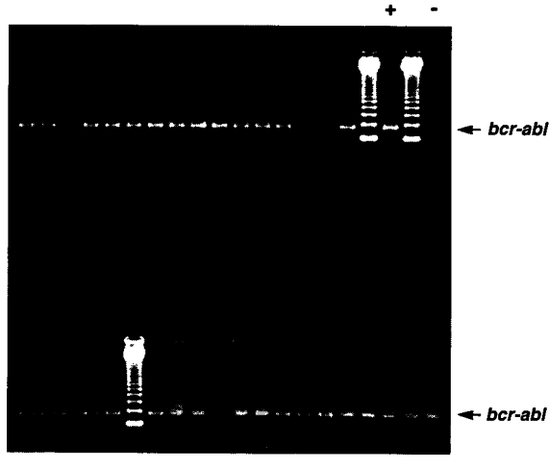

Fig. 1. Detection of *bcr-abl* in agar colonies using PCR. Each lane represents analysis of an individual agar colony. DNA from each agar colony was prepared and analyzed by PCR as described (*22*). The presence of the *bcr-abl* gene was evaluated with specific oligonucleotides that amplify a unique 200-bp fragment of this gene. Samples were subjected to 45 cycles of amplification according to the following protocol: 30 s at 93°C, 30 s at 59°C, and 15 s at 72°C. One-fifth of each reaction was run on a 2.5% agarose gel, and the specific amplified product was detected as a single ethidium bromide–stained band. The gel contains two rows of samples; arrows mark the migration of the *bcr-abl*–specific band. The identity of the band was confirmed by DNA hybridization analysis with a ^{32}P-labeled oligonucleotide specific to an internal region of the amplified *bcr-abl* segment. +, 10 ng of genomic DNA from a *bcr-abl*–transformed lymphoid cell line; −, 10 ng of genomic DNA from a nontransformed mast cell line.