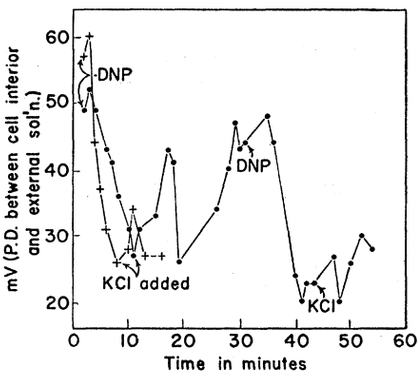

Fig. 2. Data from two experiments, showing depression of cell potential difference by dinitrophenol. In one experiment (solid points), reversal of DNP inhibition may be noted. (*Avena* coleoptile; KCl concentration, 25 mmole/lit.; DNP concentration, 0.2 mmole/lit.)

studied in the parenchyma cells of the coleoptiles. The tissue was excised and soaked in 0.2*M* mannitol (*9*) for 1 to 2 hours before the experiment, then mounted in the holder. With the electrode in a single cell, the tissue was successively perfused with 0.1, 1, 10, and 100 m*M* solutions of KCl, made up to 0.2*M* with mannitol. With the electrode still in the cell, the series was also run in reverse. Readings were recorded after each change in concentration when the potential had stabilized (a matter of seconds). As is shown in Fig. 1, the potential becomes less negative as the external concentration of KCl is increased and, conversely, more negative as the external concentration is subsequently decreased. The reversibility of the potential is a good indication that the seal around the electrode tip was maintained throughout the experiment. It is apparent that the slope is not the same as that expected of a K^+ diffusion cell see the theoretical line in Fig. 1). However, potential difference is obviously related to external KCl concentration and interpretation of the significance of slope must await further data on equilibrium conditions.

The effect of 2,4-dinitrophenol (DNP) on the cellular potential was also studied with parenchyma cells of *Avena* coleoptiles. In this case the tissue was excised and soaked in 25 m*M* KCl for 1 to 2 hours before it was mounted. Readings were taken on a single cell while the tissue was being perfused with 25 m*M* KCl and with 25 m*M* KCl plus 0.2 m*M* dinitrophenol. Figure 2 shows results of two experiments; in one the potential dropped to about one-half of its resting value in 6 minutes when dinitrophenol was added, and it did not recover when DNP-free KCl was added. The other curve is similar except that the potential jerkily returned to nearly its resting level when KCl was added. This potential was again depressed by dinitrophenol, but it did not recover a second time. It is known that dinitrophenol is a strong inhibitor of salt uptake by plant tissue (*10, 11*), and the magnitude of its effect appears to be correlated with metabolic activity (*11*). Therefore the depression of cell potential difference by dinitrophenol suggests that the potential may be directly or indirectly dependent on metabolic activity; however, an effect of dinitrophenol on permeability properties of cell membrane structure could possibly give a similar effect.

Attempts were made to ascertain the location within the cell of the potential difference. In root-hair cells of *Avena* at a stage of incipient hair elongation, a thick layer of cytoplasm is clearly visible, and it is thus possible to tell whether the electrode is in the cytoplasm or in the vacuole. The potential difference was measured when the electrode was inserted slowly into the cytoplasm and when it entered the vacuole. It was found that a major rise in potential occurred when the electrode entered the cytoplasm; the potential difference did not change significantly when the electrode entered the vacuole. Some cytoplasm-vacuole readings (in millivolts) in 10 m*M* KCl were 33/33, 30/39, 30/30, and 39/39; and in 1 m*M* KCl, 83/90, 87/84, 75/75, 78/60, and 71/71. These data, indicating that there is no difference in potential between cytoplasm and vacuole, indicate a situation similar to that in certain algae (*7*).

The existence of a constant cell potential in vascular plant tissue must be given important consideration in describing any scheme of salt absorption by plants. According to these data, the potential difference amounts to as much as 120 mv in *Avena* coleoptile cells—a difference which is sufficient to make the internal K^+ concentration more than 100 times greater than the external concentration. The location of the potential drop at the external cytoplasmic surface emphasizes the classical view of the cytoplasmic membrane as a barrier to ionic diffusion. It thus appears to definitely preclude the possibility that the vacuolar membrane is essentially the only ionic diffusion barrier and that the cytoplasm is, relatively speaking, "free space," as has been proposed (*12*).

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Characteristics of Blood-Brain Barrier to Gamma-Aminobutyric Acid in Neonatal Cat

Abstract. Systemic γ-aminobutyric acid produces a rapid, sustained, but transiently reversible blockade of evoked axodendritic excitatory postsynaptic potentials in neonatal cortex when the "barrier" operating to restrict its passage has been altered by various experimental procedures. The data obtained under these conditions indicate the existence of well-developed synaptic pathways in the superficial neuropil of immature cortex.

The functional characteristics of the blood-brain barrier in newborn animals have been analyzed by comparing the relative uptake of systemically administered dyes (*1*), metabolites (*2, 3*), and ions (*4*) by the immature and mature brain. Conflicting opinions concerning the development of this "barrier" at birth derive largely from the use of different test substances, which presumably measure different aspects of blood-brain barrier activity (*5*).

The functional characteristics of the blood-brain barrier in newborn animals (kittens) were studied with a pharmacologically active metabolite, γ-aminobutyric acid. The latter, when applied directly to exposed cortex, rapidly

eliminates surface-negative components of evoked cortical responses, presumably by blocking excitatory synapses on superficial extensions of vertically oriented apical dendrites of cortical neurons (*6*, *7*). However, when the metabolite is administered intravenously to mature cats, similar effects are observed only in regions of damage to the blood-brain barrier (*8*). Thus, the particular aspect of blood-brain barrier activity analyzed with systemic γ-aminobutyric acid relates to those factors which operate to restrict the passage of a pharmacologically active amino acid from plasma to cortical neuronal surfaces.

Experiments were performed on succinylcholine-paralyzed, locally anesthetized kittens a few hours to 6 weeks old. These were prepared for oscilloscopic registration of cortical-surface responses in a manner similar to that described for mature cats (*6*). Different concentrations of the amino acid in 0.5 to 1.0 ml of saline were injected via an indwelling catheter in the external jugular vein. The effects of these injections on two varieties of evoked cortical potentials were studied—the surface-negative response of cortex to local stimulation and the diphasic positive-negative response of somatic sensory cortex to stimulation of the lateral thalamus. The local superficial-negative cortical response is inferred to be an excitatory postsynaptic potential of apical dendrites in the molecular layer, whereas the specific thalamocortical response involves synaptic activation of neurons in the cortical depths (surface-positive component) and postsynaptic potentials of dendrites in the superficial regions of the cortex (surface-negative component) (*7*).

The results of this investigation, summarized in Fig. 1, indicate that the processes which limit or prevent diffusion of blood-borne γ-aminobutyric acid to cortical synaptic sites on apical dendrites in mature animals also operate in newborn kittens. Concentrations of the amino acid which rapidly eliminate superficial-negative cortical responses evoked in regions of blood-brain barrier destruction in mature cats produced relatively insignificant effects on such responses in neonatal animals (Fig. 1*A*). Rapid injection of high concentrations of γ-aminobutyric acid (500 mg/kg) transiently, but significantly, decreased these axodendritic excitatory postsynaptic potentials (Fig. 1*B*). Of considerable interest was the finding that the blood-brain barrier to γ-aminobutyric acid in neonatal animals could be markedly altered by exposure of the cortex for short periods (30 to 45 minutes) despite the fact that the cortex was covered with warm mineral oil. When these alterations developed, previously ineffective concentrations of γ-aminobutyric acid rapidly abolished the superficial-negative cortical response, spontaneous recovery occurring after 20 to 30 minutes (*2*). Similar effects of systemic γ-aminobutyric acid were also observed in newborn kittens injected with large amounts of the amino acid (300 to 400 mg/kg) 2 to 3 hours prior to craniectomy (Fig. 1*D*). Changes in blood-brain activity induced by prolonged exposure of the cortex or by preloading with γ-aminobutyric acid, or by both, in neonatal kittens were not similar to those produced in mature cats with lipid solvent-induced barrier lesions (*8*). In newborn kittens, the cortical synaptic effects of systemic γ-aminobutyric acid were rapidly reversed at

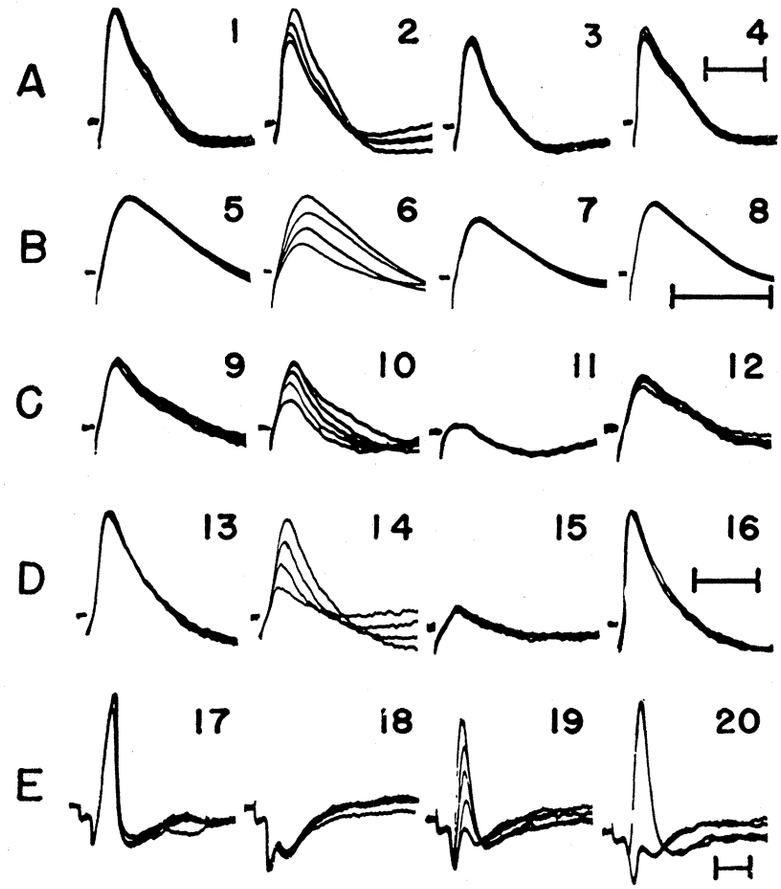

Fig. 1. (*A–D*) Superficial cortical responses in cat, recorded monopolarly (negativity, upwards) 1 to 1.5 mm from surface-stimulating wire electrodes on suprasylvian gyrus. (*E*) Posterior sigmoid gyrus responses to lateral thalamic stimulation (four to ten superposed traces throughout). Time calibrations, 50 msec; that for *C* same as for *B*. (*A*) Response in a kitten 15 hours old: (1) 10 minutes after craniectomy; (2) 10 to 18 seconds after injection of 150 mg of γ-aminobutyric acid per kilogram; (3 and 4) 1 minute and 3 minutes, respectively, after (2). (*B*) Response in a kitten 8 hours old: (5) 15 minutes after craniectomy; (6) 6 to 30 seconds after injection of 500 mg of the amino acid per kilogram; (7 and 8) 4 and 6 minutes, respectively, after injection. (*C*) Response in a kitten 3 days old: (9) 1.5 hours after craniectomy; (10) 6 to 10 seconds after injection of 100 mg of the amino acid per kilogram; and (11) 10 to 20 seconds after injection. (12) Record 30 minutes later, showing spontaneous recovery. (*D*) Response in kitten 4 days old: (13) 20 minutes after craniectomy and 2.5 hours after injection of 350 mg of the amino acid per kilogram; (14) 6 to 12 seconds after injection of 100 mg/kg; and (15) 3 minutes later. (16) Record showing immediate recovery of control responses by rinsing the cortex with Ringer's solution. (*E*) Record of thalamocortical responses in a kitten 12 hours old: (17) 2 hours after craniectomy and 4 hours after injection of 400 mg of the amino acid per kilogram; and (18) 1 minute after complete abolition of the surface-negative component by injection of 100 mg/kg. (19) Record showing immediate restoration of surface negativity by brief rinsing of the cortex with Ringer's solution, then gradual reappearance of the blockade. (20) Record showing a repeat of the recovery-blockade sequence, as in (19).

the height of synaptic blockade by flushing the cortical surface with Ringer's solution (Fig. 1, *D* and *E*). Synaptic blockade again developed in 30 to 60 seconds when rinsing was discontinued, presumably due to a continued influx of the amino acid (Fig. 1*E*). This sequence (blockade; recovery by surface application of Ringer's solution; blockade), once established, was reproducible for 20 to 40 minutes. At the end of this period no further reduction in surface-negative components of evoked responses occurred after a brief rinsing of the cortex. Unequivocal synaptic effects of systemic γ-aminobutyric acid resulting from alterations in blood-brain barrier activity induced by relatively short periods of exposure of the cortex or by preloading with the amino acid were regularly observed in kittens less than 10 days old and were rarely seen 2 to 3 weeks postnatally.

The presence in newborn cats of a blood-brain barrier to a pharmacologically active metabolite is suggested by the observations that single injections of γ-aminobutyric acid do not effectively alter cortically evoked responses until some change has occurred, presumably in the interposed elements between plasma and neurons which constitute this barrier. Then, influx of γ-aminobutyric acid is signaled by a blockade of axodendritic excitatory postsynaptic potentials that is rapidly reversed during and immediately after surface application of Ringer's solution. Rapid reversibility, as illustrated in Fig. 1, *D* and *E*, is seen after topical application of the amino acid (*6*) but has not been observed in mature cats with experimentally induced lesions of the blood-brain barrier (*8*). Apart from any differences in the degree of blood-brain barrier damage which may have been produced by lipid solvents in mature animals and by exposure of the cortex in the newborn kitten, differences in reversibility may be attributable to the presence of a greater number of subsurface axodendritic synapses in the mature cortex which are blocked by systemic γ-aminobutyric acid but are inaccessible to Ringer's solution applied to the surface. The rapid reversibility of systemic γ-aminobutyric acid effects observed in the immature animal emphasizes the superficial location of the axodendritic synapses blocked by the amino acid. This indicates the existence of well-developed synaptic pathways in the superficial neuropil of immature cortex at a time when its spontaneous electrical activity is poorly organized.

Little information has been obtained concerning the nature of the processes which operate to prevent blood-borne γ-aminobutyric acid from gaining access to cortical neuronal surfaces, or why these mechanisms are so labile in the neonatal period. The relative ease with which functional changes in the blood-brain barrier to injected γ-aminobutyric acid are produced in newborn kittens may be a consequence of the incomplete development of perivascular glial elements at birth (*9*). Since it has been shown that in mature animals the blood-brain barrier restricts net uptake but not rapid exchange of some amino acids between plasma and brain amino acid pools (*10*), a process may be envisoned whereby changes in the activity of poorly developed amino acid transport mechanisms in "immature" glial elements could result in the rapid and sustained influx of γ-aminobutyric acid demonstrable in the neonatal animal. Alternative hypotheses based on postulating different permeability properties of capillary-glial membranes at different stages of maturation may also account for the changes in blood-brain barrier activity resulting from short periods of exposure of the cortex. At present, however, it is difficult to interpret the effects of loading with γ-aminobutyric acid or of subsequent test-injections of the amino acid exclusively in terms of changes in membrane permeability (*11*).

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Effects of Veratrine and Cocaine on Cerebral Carbohydrate-Amino Acid Interrelations

Abstract. The rate of aerobic transformation of glucose-U-C^{14} into radioactive amino acids by slices of rat-brain cortex is greatly influenced by the K^+/Ca^{++} ratio in the incubating medium. Protoveratrine has effects on the amino acid pattern resembling that due to an increase in the K^+/Ca^{++} ratio. These effects are antagonized by cocaine and may be correlated with the neurophysiological activities of these drugs.

We have shown (*1–3*) that the conversion of uniformly (C^{14}) labeled glucose into radioactive amino acids (glutamic acid, glutamine, gamma aminobutyric acid, aspartic acid, and alanine) in the presence of slices of rat-brain cortex involves processes whose rates are markedly influenced by the K^+/Ca^{++} ratio in the medium bathing the slices and by the presence of a respiratory inhibitor such as malonate or of small concentrations of a narcotic such as Amytal. It was demonstrated that the changes in relative yields of the radioactive amino acids derived from glucose-U-C^{14} brought about by the presence of an increase in K^+ concentration, and by the presence of malonate, may be satisfactorily explained on the basis of the conclusions that the amino acids are derived from glucose by transamination of the alpha ketonic acids obtained during the operation of the citric acid cycle in the brain cell and that the potassium ion stimulation of brain cortex metabolism is due to an acceleration of a pace-making step, the oxidation of pyruvate by diphosphopyridine nucleotide to acetyl-CoA. It was further demonstrated that the effects of the presence of small concentrations of the narcotic Amytal on the relative yields of radioactive amino acids are adequately explained on the basis of the conclusion that the main effect of the narcotic is to suppress the oxidation of reduced diphosphopyridine nucleotide by cytochrome oxidase and its associated phosphorylations.

It is shown in the results described in this report that the presence of cocaine and of protoveratrine has effects on the relative yields of radioactive amino acids from glucose-U-C^{14} by slices of rat-brain cortex which throw light on the mode of action of these drugs and which may be correlated with their known neurophysiological effects.

The experimental work was carried out by the method we have described (*2*). Slices of rat-brain cortex were allowed to respire in oxygen at 37°C for 60 minutes in 1 ml of Krebs-Ringer phosphate medium, *p*H 7.4, containing