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Cerebral cortical blood flow maps are reorganized in MAOB-deficient mice

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Abstract

Cerebral cortical blood flow (CBF) was measured autoradiographically in conscious mice without the monoamine oxidase B (MAOB) gene (KO, $n = 11$) and the corresponding wild-type animals (WILD, $n = 11$). Subgroups of animals of each genotype received a continuous intravenous infusion over 30 min of phenylethylamine (PEA), an endogenous substrate of MAOB, ($8 \text{ nmol g}^{-1} \text{ min}^{-1}$ in normal saline at a volume rate of $0.11 \text{ } \mu\text{l g}^{-1} \text{ min}^{-1}$) or saline at the same volume rate. Maps of relative CBF distribution showed predominance of midline motor and sensory area CBF in KO mice over WILD mice that received saline. PEA enhanced CBF in lateral frontal and piriform cortex in both KO and WILD mice. These changes may reflect a differential activation due to chronic and acute PEA elevations on motor and olfactory function, as well as on the anxiogenic effects of this amine. In addition to its effects on regional CBF distribution, PEA decreased CBF globally in KO mice (range -31% to -41% decrease from control levels) with a lesser effect in WILD mice. It is concluded that MAOB may normally regulate CBF distribution and its response to blood PEA.

Keywords

Cerebral blood flow; Monoamine oxidase; Phenylethylamine; Autoradiography; Iodo-antipyrine; Mouse

1. Introduction

Monoamine oxidases A and B are isoenzymes that act on endogenous and exogenous bioamines. Phenylethylamine (PEA), an endogenous amine that can also be found in certain foods, is a specific substrate of monoamine oxidase B (MAOB). PEA has been proposed to be a possible mediator in the regulation of cerebral blood flow since it can alter cerebral perfusion in experimental animals [33]. In contrast, most other monoamines (e.g., serotonin, noradrenaline, tyramine) show extremely low penetration of the blood–brain barrier [37] and do not effectively alter cortical blood flow (CBF) when administered peripherally at moderate doses [31].

Enzymatic degradation by MAOB plays a central role in regulating tissue levels of PEA. Within the circulation, access of PEA to cerebral vessels and brain tissue depends to a large extent on MAOB found in platelets, as well as on MAOB in the astrocytic foot processes and capillary endothelial cells that constitute the blood–brain barrier [24]. The physiological significance of MAOB and PEA in the control of CBF remains unknown. Grimsby et al. [18] have recently shown that targeted inactivation of MAOB in mice results in an eight-fold increase in levels of PEA in brain but not of serotonin, norepinephrine or dopamine. These mice offer a unique opportunity to test for a role of PEA in the control of the cerebral circulation.

We examined CBF measured with the autoradiographic iodo- ^{14}C -antipyrine (IAP) method in MAOB knockout mice (KO) and compared it to wild type (WILD) controls. We also analyzed the effect of intravenous (i.v.) PEA infusion on the CBF of KO and WILD mice. The high spatial resolution of the autoradiographic technique allows detailed mapping of cortical CBF. When this data is transformed to the standard normal deviate (CBF Z-scores), Z-maps are obtained. Such maps allow examination of the regional variations created by differential neuronal activity, while excluding variations in average CBF between subjects and experimental groups created by global effects on vascular smooth muscle and systematic experimental error. This approach has been exploited extensively in the clinical literature for the localization of brain function [5]. In its application to the present material, we hoped to reveal patterns of cortical activity in mice that could be modulated by absence of MAOB and/or administration of its endogenous substrate, PEA. We have also analyzed the non-transformed CBF values, that may provide additional information on global effects of these interventions, possibly mediated by direct action on cerebral vascular smooth muscle.

2. Methods

2.1. Animals

Adult, male MAOB-deficient mice (KO) of C57Bl-6r129Sv genetic background [18] and wild-type littermates (WILD) were housed four per cage at the Animal Care Facility at the University of Southern California. One week prior to experimentation the animals were transferred to the Animal Care Facility at the West Los Angeles Veterans Administration Medical Center. Here they were maintained ad libitum on water and rodent laboratory chow in an environmentally controlled room on a 12-h light, 12-h dark cycle (lights on at 0700 h).

All procedures performed were reviewed and approved by the Animal Care and Use Committee at the West Los Angeles Veterans Administration Medical Center. At the time of the experiment animals were matched by age and weight (WILD: $n = 11$, 6.0 ± 0.1 months, 43 ± 2 g; KO: $n = 11$, 6.0 ± 0.1 months, 43 ± 2 g). At the end of the experiment, the genotypes of the mice were re-confirmed using a polymerase chain reaction (PCR) analysis of DNA prepared from tails [18].

2.2. PEA infusion

Animals were anesthetized with halothane (2.5% for induction, 1.0% for maintenance in 30% oxygen, 70% nitrous oxide) and received cannulation of the femoral artery and vein. A 1-h period was allowed for recovery from anesthesia while the animal movements were limited by flexible paper restraints linking the limbs to a padded surface. Rectal temperature was recorded and maintained at 37°C with a BAT-12 thermocouple thermometer connected to a TCAT-1A temperature controller (Physitemp) and a source of radiant heat. Following recovery from anesthesia mean arterial blood pressure (MABP) and heart rate were assessed from the arterial catheter which was connected to a pressure transducer and polygraph recorder. Animals of each genotype received a continuous intravenous infusion over 30 min of PEA (WILD/PEA: $n = 5$, KO/PEA: $n = 5$) or saline (WILD/SAL: $n = 6$, KO/SAL: $n = 6$). The dose of PEA ($8 \text{ nmol g}^{-1} \text{ min}^{-1}$ normal saline at a volume rate of $0.11 \text{ } \mu\text{l g}^{-1} \text{ min}^{-1}$) was taken from that found by McCulloch and Harper [34] to be effective in baboons after adjustment for equivalence between species [12]. Infusion ended following the assessment of CBF, a terminal procedure.

2.3. Assessment of CBF

Immediately prior to implementing the CBF technique, arterial blood gases and pH were assessed from an arterial blood sample (70 μl) in a Radiometer ABL-30 blood acid–base system. Assessment of CBF was made with a modification of the indicator fractionation method using ^{14}C -iodo-antipyrine (^{14}C -IAP) [17]. Cerebral concentration of this tracer was determined with quantitative autoradiography as described below. CBF was calculated from the following equation:

$$CBF = \frac{C_b(T)}{\int_0^T C_a(t) dt}$$

where C_b is the brain tissue concentration of tracer at time T (decapitation), and C_a is the arterial tracer concentration. Integration of the arterial concentration time curve was performed mechanically by continuous withdrawal of blood at a constant rate.

Animals received a continuous intravenous infusion over 10 s of 180 μl saline containing 270 $\mu\text{Ci kg}^{-1}$ b.wt. of ^{14}C -IAP (American Radiolabelled Chemicals) administered by a motor-driven syringe pump. Arterial blood was continuously withdrawn at a rate of 15 $\mu\text{l s}^{-1}$, from 2 s before the beginning of tracer administration until 10 s after this event, when the animal was decapitated. This procedure was adopted to balance the infusion and withdrawal of fluids and avoid sudden changes in arterial volume and pressure given the small blood volume of the mouse. The arterial blood sample was processed for liquid

scintillation counting of radioactivity in a Beckman LS8100 liquid scintillation spectrometer. Counting efficiency was verified by internal standardization with ^{14}C -hexadecane.

2.4. Autoradiography and data analysis

The brains were rapidly removed, flash frozen in methyl-butane at -70°C , embedded in OCTTM compound (Miles) and stored at -70°C . Brains were subsequently cut in a cryostat at -16°C in 20 μm -thick coronal sections. Sections were heat-dried on glass slides and exposed for 2 weeks to Kodak Ektascan films in spring-loaded X-ray cassettes along with eight radioactive ^{14}C standards (Amersham). Images (autoradiographs) of brain sections were digitized with a ChromaPro 45 IAIS 'Dumas' film illumination system and a Phillips Charge Coupled Device (CCD) monochrome imaging module coupled to an AT and T Targa M8 digitizing board on a Tandon PCA/12 microcomputer. Brain regions were identified on autoradiographs using an anatomic atlas of the mouse brain [11]. The optical density of locations in the cortical mantle, was measured with Image Pro-Plus software (Media Cybernetics). Quantification of optical density of autoradiograms and comparison with that induced by standards of known radioactivity allowed determination of ^{14}C activity and CBF. Measurement of regional CBF were performed in 105 cortical regions, distributed in 11 coronal planes as defined in Fig. 1.

For statistical analysis each animal's CBF was averaged by region for the left and right hemispheres. For every experimental group means and standard errors of the means (S.E.) were calculated for each region, each slice (all regions in a given slice), and globally (all regions in all slices). CBF group differences in slice and global averages of WILD/PEA, KO/PEA and KO/SAL vs. the control (WILD/SAL) were assessed by analysis of variance (ANOVA) and *t*-tests (unpaired, two-tailed, $P < 0.05$) using the Bonferroni correction for three contrasts.

In addition to the univariate approach described above, multivariate analysis of patterns of relative CBF distribution was performed. These patterns (Z-maps) were defined by calculation of standard normal deviates (Z-scores) defined as:

$$Z - \text{score}_i = \frac{(CBF_i - \text{Mean})}{\text{S.D.}}$$

where CBF_i is the blood flow of location i , Mean and S.D. are the average, and standard deviation of blood flow at all locations in a given animal, and $Z\text{-score}_i$ is the standard normal deviate of blood flow at location i . This transformation has been proposed for the analysis of positron emission tomography maps because it introduces minimal dependence on absolute CBF when the number of locations studied is large, as in the present case, and it does not propagate further error [5]. Display of Z-scores in a three-dimensional map, where the x - and y -axis correspond to the geometry of the cortical surface and the z -axis represents a difference in mean Z-scores between experimental groups for every location (Fig. 1), allows rapid appreciation of the magnitude and spatial organization of differences in CBF patterns. Abstraction of the CBF pattern by this procedure eliminates the differences in mean CBF

between subjects and experimental groups. This is an advantage when studying patterns, but it precludes discovery of potential differences in mean rates. We have consequently performed both univariate and multivariate analysis of CBF.

Z-scores were then analyzed by stepwise discriminant analysis [23] to reduce the 105 blood flow variables (cortical locations) to a smaller set that optimally discriminates among all the experimental groups. The discrimination obtained with this analysis is represented in a scatter plot (canonical plot, Fig. 2), with the first canonical variable (the linear combination of variables that best discriminates among the groups) on the abscissa, and the second canonical variable (the next best linear combination orthogonal to the first one) on the ordinate. Group classification was repeated using the 'jackknife' procedure. This involves leaving out each of the cases in turn, calculating the function based on the remaining $n - 1$ cases, and then classifying the left-out case. Since the case which is being classified is not included in the calculation of the function, the observed classification rate is a less biased estimate of the true one. Characteristic profiles, representing patterns of blood flow that uniquely correspond to each of the canonical variables were computed using an approach described in detail elsewhere [42].

3. Results

3.1. Cortical blood flow

WILD and KO mice differed in the topographical distribution of CBF Z-scores during the saline infusion. WILD mice revealed their highest CBF Z-scores in a band of cortex extending from the motor area anteriorly, to the auditory and secondary visual areas posteriorly, including the somatosensory and barrel field regions. An additional cluster of locations with high CBF Z-scores was found also in the anterior piriform cortex. KO mice that received saline duplicated this pattern with the addition of enhanced CBF Z-scores in the motor and sensory areas closest to the midline. The difference between the two maps (KO/SAL – WILD/SAL) is shown in Fig. 1, top panel. Administration of PEA induced a cluster of enhanced CBF Z-scores in the anterior piriform and lateral frontal cortex (primary motor and primary somatosensory cortex) in both WILD and KO mice. This phenomenon is shown for KO mice in Fig. 1, middle panel (KO/PEA – KO/SAL).

Z-maps were studied by stepwise discriminant analysis. This procedure achieved 100% success in classifying all cases into their respective groups, with data from 15 cortical locations selected by the discriminant procedure from the original 105 locations (Table 1). After 'jackknifing', a procedure that removes from the discriminant function the contribution of the case being classified, the effectiveness of classification remained at 100% for all animals, except those receiving PEA infusions in which correct classifications dropped to 80% due to overlap among the WILD/PEA and KO/PEA groups in two cases out of 10.

The first two canonical variables explained 97.9% of the total variation and they allowed representation of canonical scores for each case in a single plane (canonical plot, Fig. 2). Examination of this plot indicates that our experimental groups are distinct, with tight clustering of cases within groups. The first canonical variable separated the PEA from the

saline treatment groups (PEA variable), and the second one separated the mice with different genotypes (genotype variable). The displacement of both variables along the axes of the canonical plots depends on the values of both the characteristic profile (Table 1) and the CBF Z-scores.

For the PEA variable, characteristic profile values were large and negative for piriform cortex (PI), secondary somatosensory (S2), barrel field (BF) and secondary motor (M2) regions, as well as the transitional zone (TB) between primary somatosensory (S1) and primary motor (M1) regions. This explains the negative shift on the canonical plot of the mice that received PEA as these animals had greater CBF Z-scores in these regions. Perirhinal (PR), retrosplenial (RS) and M1 regions had positive characteristic profile values for the PEA variable, and since CBF Z-scores were negative in these areas, this also contributed to the left shift in the PEA groups.

Characteristic profile values of the genotype variable were most negative in M1 and S1 areas, as well as in the transition cortex (TA) between the barrel field and S2 cortex, and in transition cortex (TD) between cingulate and M2 cortex. Profile values of the genotype variable were positive in piriform (PI) and S2 areas, as well as in the transitional areas (TB) between M1 and S1 cortex, and in the transitional areas (TC) between secondary somatosensory and perirhinal cortex. The positive CBF Z-scores observed in the regions with negative characteristic profiles, and the negative CBF Z-scores found in the areas with positive characteristic profiles explains displacement of KO mice towards negative values on the ordinate of the canonical plots.

PEA infusion compared with saline infusion resulted in a decrease in absolute CBF ($\text{ml g}^{-1} \text{min}^{-1}$) in both WILD and KO mice. Statistical significance was attained in CBF slice averages of KO/PEA mice when compared to WILD/SAL mice ($P < 0.05$, Bonferroni correction for three contrasts) (Fig. 3). No difference in slice averages of CBF was found between WILD/SAL and either WILD/PEA or KO/SAL mice.

Global CBF was also significantly lower ($P < 0.05$, Bonferroni correction for three contrasts) in KO mice that received PEA ($0.95 \pm 0.08 \text{ ml g}^{-1} \text{min}^{-1}$) than in WILD mice that received saline ($1.76 \pm 0.22 \text{ ml g}^{-1} \text{min}^{-1}$). No difference in global CBF was found between WILD/SAL and either WILD/PEA mice ($1.41 \pm 0.30 \text{ ml g}^{-1} \text{min}^{-1}$) or KO/SAL mice ($1.53 \pm 0.19 \text{ ml g}^{-1} \text{min}^{-1}$).

3.2. Effects on physiological variables

KO and WILD mice showed no significant change in mean arterial pressure (MABP), heart rate, body temperature, arterial blood gases or pH (Table 2) in response to saline infusion. PEA infusion resulted in a significant increase in heart rate in both the WILD and KO mice, and a significant increase in MABP in the KO mice ($P < 0.05$, after Bonferroni correction). Both groups showed an increase in body temperature in response to PEA, with statistical significance reached in WILD mice. Blood PCO_2 was significantly lower in KO mice receiving PEA when compared to KO mice receiving saline (Table 2). The same trend was observed in WILD mice although with no statistical significance.

4. Discussion

Normalized CBF cortical maps (Z-maps) indicated striking differences between both mouse genotypes, with predominance of CBF in midline motor and sensory cortex in KO over WILD mice. PEA administration induced enhancement of CBF Z-scores in piriform and lateral frontal cortex of both KO and WILD mice. These differences in the Z-maps may reflect a differential degree of activation of the cerebral cortex derived from the effects of chronic and acute PEA elevation. In addition, a global effect on CBF was also present following PEA administration as demonstrated by generalized decreases in non-transformed CBF. MAOB may regulate the CBF response to PEA in vivo, since the global decrease in CBF observed with PEA administration was greater in KO mice.

CBF Z-scores showed a relative increase in motor and sensory areas in mice lacking the MAOB gene. These animals have an excess of brain PEA [18], and exogenous administration of this amine is known to increase motor activity and motor stereotypies [2]. Previously, however, we have found that locomotor activity is not enhanced in these animals in the open-field test [18], so it is conceivable that compensatory mechanisms may have developed to block the expression of this behavior while the overactivity of motor cortex may still be present.

Administration of PEA induced similar changes of CBF Z-maps in WILD and KO mice (shown for KO mice in Fig. 1, middle panel), with an increase of CBF Z-scores in piriform and lateral frontal cortex. This effect of PEA bears no resemblance to the genotype effect and suggests that the acute effects of excess PEA on cerebral cortical activity are essentially different from those of chronic exposure to elevated PEA levels.

The cluster of increased CBF Z-scores in piriform cortex invites speculation. This cortical area (primary olfactory area) receives input from the olfactory bulb and is essential for learning involving olfactory cues [45] in which this cortical area may cluster similar co-occurring odors [36]. Lesion of the piriform cortex blocks olfactory-related behaviors such as learning of a navigational map in homing pigeons [14] and pup seeking (parental) behavior in mice [27]. Moreover, a focal increase of CBF in piriform cortex has been demonstrated with odor stimulation in humans [28], and degeneration of neurons in piriform cortex is characteristic of patients with Alzheimer's disease, which are known to be unable to perform tasks involving discrimination of new odors [25]. Another possible function proposed for piriform cortex has been in the mediation of anxiety. Acute administration of PEA is known to induce anxiety similar to that induced by its analog amphetamine [29], which has been reported to activate piriform cortex and other allocortical areas in a model of sensitization [19]. That behavioral effects of chronically elevated PEA levels may differ from those following acute PEA administration is suggested by the fact that KO mice do not show increased anxiety in the elevated plus maze [18]. We assume that the regional variations in CBF discussed above are due to regional variations in neuronal activity since direct effects on vascular smooth muscle are most likely global in nature and would be eliminated by the Z-transformation used. The possibility of regional variations in the effect of PEA on vascular smooth muscle cannot be completely discarded, however, and may require further experimentation, such as measurements of regional metabolism.

Like most other gene knockout mice, MAOB-deficient animals were a hybrid of two strains. It is possible that this mixture of strains could contribute to differences in the topography of CBF between KO and WILD mice. Future studies may wish to examine CBF in animals that have been backcrossed to a pure background.

The study of non-transformed CBF data revealed that PEA induced absolute decreases in CBF that were more marked in KO mice, and occurred in a global distribution. These results are in line with earlier work by McCulloch and Harper [33,34] demonstrating that MAOB may regulate the CBF response to acute administration of PEA. These authors, using the ^{133}Xe technique in anesthetized baboons, found a 28% decrease in CBF following intracarotid administration of PEA ($2 \mu\text{mol kg}^{-1} \text{ min}^{-1}$). The relatively small global decrease in absolute CBF observed in KO mice, despite markedly elevated tissue levels of PEA [18], again may represent a physiologic adaptation in these mice to a lifelong absence of MAOB. In theory, such an adaptation might occur within alternate metabolic pathways such as MAOA, aldehyde dehydrogenase [13], and dopamine beta-hydroxylase [7] which under normal physiologic conditions account for only a small proportion of PEA catabolism.

The mechanism of action of PEA in effecting changes in CBF by direct action on vascular smooth muscle remains unknown. When tested on resistance vessels in vitro, PEA increases smooth muscle tone and enhances stimulation induced contractions by a noradrenaline displacing effect [26]. In the central nervous system, it has been suggested that PEA has a relatively potent effect inhibiting the uptake of dopamine, norepinephrine and possibly serotonin [20,40]. In addition, PEA stimulates the release of the catecholamines and to a lesser extent serotonin [1,8,40]. Previous work, however, has shown no significant effect of phenoxybenzamine, an alpha adrenergic antagonist, or pimozide, a dopamine antagonist on PEA-induced changes in CBF [34]. Such evidence suggests that dopaminergic or noradrenergic receptor activation is not involved in the PEA-induced cerebrovascular response. A possible interference with central cholinergic transmission may be a factor, since it is known that PEA depresses acetylcholine contents of cerebral cortex [21]. Alternative explanations, such as changes in CBF resulting from the action of serotonin or such metabolites of PEA as phenylethanolamine need to be considered.

PEA administration resulted in a small elevation of MABP in KO mice but not WILD mice, likely because KO mice lack the necessary enzyme to effectively metabolize PEA. As stated above, PEA enhances the release of noradrenaline and dopamine from sympathetic nerve endings and may, thus, induce elevation of systemic vascular resistance and also enhance myocardial contractility. In fact, dose-dependent increases in MABP, total peripheral resistance, and left ventricular P/t have previously been reported following acute PEA administration [30]. Pretreatment with phentolamine, an α -adrenergic antagonist, reduces the increases in aortic blood pressure and peripheral vascular resistance, while the effect on P/t is blocked by propranolol, a β -adrenergic antagonist [30]. In addition to peripheral mechanisms, the involvement of a central augmentation of sympathetic tone is likely, in view of the prominent effects of PEA on central adrenergic transmission [26,38,39], as well as behavioral arousal [29]. Thus, a pressor response following PEA may be the complex result of an enhanced central nervous system sympathetic out-flow and peripheral augmentation of noradrenergic action at the heart and blood vessels. The pressor response to

PEA was within the autoregulation limits of CBF, and thus, was not expected to induce changes in this variable. This was confirmed by observation of a plot of CBF as a function of MABP (not shown) which revealed no trends on CBF within the observed range of MABP.

The hyperthermic effect of PEA observed in the present experiments is in line with previous observations in dogs [43]. The fact that the effect was similar in KO and WILD mice suggests that MAOB may not be the limiting factor for the expression of this phenomenon. The enhancement of heart rate, also observed after PEA infusion in both groups of mice, may have been due, at least in part, to the hyperthermia. The fact that changes in these variables in response to PEA were similar in both genotypes rules out their role in the differential effect of this amine on CBF of KO and WILD mice. Although blood PCO_2 showed a small decrease in KO mice following PEA, no significant change in blood pH was found. Thus, it is unlikely that this change may have influenced CBF. As in the case of MABP, no trends in CBF were observed when this variable was plotted as a function of blood PCO_2 .

Cerebrovascular changes are a prominent feature of migraine headache, with vasoconstriction of intracranial blood vessels provoking a decrease in blood flow and concomitant neurologic signs [6], followed by a prolonged dilation of the extracranial arteries, which provokes the pain. Oral ingestion of small amounts of PEA by migraine patients can provoke headaches that are similar to their usual attacks, whereas normal subjects tolerate large amounts of PEA by the same route without untoward effects [41]. Likewise, foods such as chocolate, cheese and wine with a high PEA content [32] are amongst the factors that may trigger migraine headaches in susceptible individuals [48]. It has been hypothesized that reduced MAOB activity in migraine sufferers may increase their susceptibility to concentrations of PEA that in normal individuals result in no untoward effects [41]. In theory, lower levels of MAOB in the peripheral circulation should result in a higher fraction of PEA that reaches cerebral vessels. Several investigators have reported a 20–40% decrease in platelet MAOB activity in subjects with migraine headaches [3,4,22,41,44,49], as well as decreases in MAOB activity during the course of an acute episode [16,44]. Such findings, however, remain controversial with some authors reporting decreases only in a subpopulation of migraine patients [15], and others reporting ‘increases’ [9] or ‘no change’ [35,46]. Differences may relate to factors including cigarette smoking [10,49] and hormonal status [9,47] that independently affect MAO levels and have not been accounted for in most studies.

Our study suggests that PEA is active in the modulation of cerebral perfusion, an effect which is accentuated in animals lacking MAOB. The possibility that low levels of MAOB may represent a risk factor in vasospastic syndromes including migraine needs to be considered.

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Fig. 1.

Bars represent Z-score differences between KO mice and WILD mice receiving saline (top figure), and KO mice receiving saline or PEA (middle figure). Negative values are shown with bars projecting below the zero plane (black top). Coronal slices are numbered from rostral to caudal, with distance to bregma as follows (positive values being rostral to this landmark): (1) 1.54 mm, (2) 1.18 mm, (3) 0.74 mm, (4) 0.26 mm, (5) -0.34 mm, (6) -0.82 mm, (7) -1.34 mm, (8) -1.82 mm, (9) -2.46 mm, (10) -2.92 mm, (11) -3.64 mm. The cerebral cortical locations corresponding to each measurement are numbered from dorsal midline to lateral and ventral. These are shown in the bottom panel. Abbreviations: AI = agranular insular, AU = auditory, BF = barrel field, C1 = cingulate, CA = amygdaloid, EC = ectorhinal, EN = entorhinal, GI = granular insular, M1 = primary motor, M2 = secondary motor, PI = piriform, PR = perirhinal, RS = retrosplenial, S1 = primary somatosensory, S2 = secondary somatosensory, TA = transition between barrel field and secondary somatosensory, TB = transition between primary somatosensory and primary motor, TC = transition between secondary somatosensory and perirhinal, TD = transition between cingulate and motor, V1 = primary visual, V2 = secondary visual. Outlined areas are those listed in Table 1.

Fig. 2. Canonical plot for the two first canonical variables providing discrimination between the relative CBF (Z-scores) of KO and WILD mice receiving saline, and by infusion type (PEA or saline) for both KO and WILD mice. For details see text.

Bregma 0.26 mm

Bregma -0.82 mm

Fig. 3.

Two representative iodo- ^{14}C -antipyrine autoradiographs of slice 4 and slice 6 are shown in the left panels. Regions sampled are indicated by numerals. The corpus callosum and anterior commissure (slice 4) and the corpus callosum and hippocampal commissure (slice 6) are outlined as reference. The bar graphs (right panels) indicate group means and standard errors of CBF of the regions (numerals), as well as the slice means (ALL) in units of $\text{ml g}^{-1} \text{min}^{-1}$. Abbreviations: WILD + S = WILD mice receiving saline; WILD + PEA = WILD mice receiving PEA; KO + S = KO mice receiving saline; KO + PEA = KO mice receiving PEA. * Statistically significant when compared to WILD + S, $P < 0.05$, Bonferroni correction for three contrasts.

Table 1

Characteristic profiles for the first two canonical variables

Region	Characteristic profiles ($\times 10^3$).	
	PEA variable	Genotype variable
AU	-1.91	-15.29
AU	5.39	-17.06
BF	-20.86	-9.14
M1	14.96	-41.36
M2	-14.69	-3.95
PIR	-33.06	20.44
PIR	-27.48	14.86
PR	20.28	-5.50
RS	29.14	-0.76
S1	-2.63	-27.52
S2	-16.70	29.80
TA	-4.20	-43.09
TB	-13.73	11.91
TC	5.52	12.74
TD	1.85	-38.18

Abbreviations of cortical areas are detailed in the legend of Fig. 1.

Table 2

Physiologic variables (Mean $\pm$ S.E.) during measurement of CBF in WILD and KO mice following challenge with phenylethylamine (PEA) or saline (SAL)

	Units	Wild saline	KO saline	Wild PEA	KO PEA
pH	$-\text{Log}_{10} [\text{H}]$	7.293 ± 0.011	7.311 ± 0.008	7.254 ± 0.018	7.239 ± 0.038
PCO_2	mmHg	47.4 ± 0.3	45.1 ± 1.6	37.4 ± 2.1	$29.7 \pm 1.5^*$
PO_2	mmHg	91.8 ± 3.9	92.1 ± 4.5	101.0 ± 5.2	107.6 ± 3.6
MABP	mmHg	98.8 ± 7.3	92.3 ± 2.9	98.6 ± 4.0	$105.5 \pm 4.9^{**}$
HR	Beats min^{-1}	584 ± 24	552 ± 21	792 ± 44	765 ± 22
Core Temp	$^{\circ}\text{C}$	37.5 ± 0.3	37.0 ± 0.16	39.4 ± 0.4	38.4 ± 0.7

* $P < 0.01$ (Bonferroni adjusted) vs. KO + saline or WILD + saline.

** $P < 0.01$ vs. measurements before PEA infusion in the same animals.