Table 4 | (A) Principal component analysis of three groups of female mice [all females, wildtype (WT) females only, and knockout (KO) females only]. For each group there were 21 principal components (PCs), but only those given below satisfied the first criterion of $\lambda^2 > 1$. Significant eigenvalues, marked as bolded numbers with asterisks, satisfied the second and third criterion. Other eigenvalues that shared similarities to significant eigenvalues in other groups are marked with a tilde (~). Both marked eigenvalues correspond to PCs that were considered in later analyses. **(B)** Rotated component matrix for groups of female mice with significant PCs derived from the principal component analysis displayed in **Table 4.** The columns below are the significant PCs; there were no insignificant PCs of shared similarity. The coefficient value for each PC is given after VARIMAX rotation.

	All ♀			♀WT only			♀ERKO only		
	Eigenvalue	**% Variance**	**Sign.**	**Eigenvalue**	**% Variance**	**Sign.**	**Eigenvalue**	**% Variance**	**Sign.**
A. PRINCIPAL COMPONENT ANALYSIS									
1	3.935	30.268	***4.786**	4.629	35.604	***2.179**	3.950	30.383	***1.529**
2	2.081	16.006	2.879	2.690	20.689	***1.901**	2.691	20.700	***1.323**
3	1.693	13.026	0.250	1.800	13.844	***0.793**	1.868	14.369	***5.974**
4	1.559	11.991	2.408	1.332	10.244	***10.342**	1.246	9.585	0.376
5	1.021	7.851	0.796	0.742	5.706	0.083	1.142	8.784	0.637
6	3.935	30.268	4.786	4.629	35.604	2.179	3.950	30.383	1.529

	All ♀	♀WT only				♀ERKO only		
	***1**	***1**	***2**	***3**	***4**	***1**	***2**	***3**
B. MATRIX NUCLEI								
LSI	−0.002	0.240	***0.771**	0.207	−0.246	−0.183	0.033	−0.036
MPOA	−0.023	−0.195	***0.875**	−0.029	−0.277	0.100	0.074	−0.007
PE	0.137	−0.139	***0.839**	−0.080	0.237	***0.721**	−0.164	0.148
ACO	***0.755**	***0.874**	0.150	0.130	−0.212	***0.775**	0.364	−0.152
MEAA	***0.885**	***0.946**	0.083	0.073	−0.006	***0.786**	0.069	−0.393
CEA	***0.858**	***0.904**	−0.138	−0.185	0.085	***0.835**	0.178	0.006
VMH	0.258	−0.113	0.229	***0.697**	−0.472	0.386	***0.831**	−0.112
LHb	−0.254	−0.146	0.311	***−0.796**	0.048	−0.290	−0.103	***0.899**
MHb	0.117	0.041	−0.109	0.060	***0.865**	0.121	0.037	***0.864**
MEAPD	***0.648**	***0.820**	−0.204	0.261	0.160	0.280	0.192	0.338
MEAPV	***0.726**	***0.873**	−0.125	0.236	0.112	0.387	***0.650**	0.246
VMHDM	−0.017	0.067	0.374	***0.727**	0.414	−0.131	***0.736**	−0.007

Asterisk bolded numbers indicate nuclei with a coefficient greater than 0.60 in significant PCs.

when raised in litters containing either ♂KO or ♂WT littermates. Anxiety-like behaviors, reflected in behavior in the Light:Dark box, were modified in WT males if they had WT sisters in the litter, an effect that was absent if the sisters were KO mice. A summary of the behavioral data obtained with females indicate that the social behavioral profile of ♀KO mice is most clearly distinguished from that of ♀WT mice when ♀KO mice are raised in litters containing only ♀KO mice; ♀KO mice raised with ♂WT mice behave similarly. ♀KO mice are more similar to ♂WT mice than they are to ♀WT mice in their behavior, metabolic activity, and functional connectivity, suggesting that ♀KO mice play a male-type role in the preweaning sibling environment.

Regarding the effect of Genotype and Sex ratios of the litter on brain metabolic activity later in adulthood, it is noteworthy that ♂WT and ♂KO do not differ in their WB metabolic activity, but when male mice were raised in litters having ♂KO, or females of either Genotype, overall brain activity was higher than if raised in litters having only WT males. In females, those having ♀WT siblings had higher WB activity, and the presence of ♀KO or ♂WT siblings had the effect of lowering WB activity. Whether this and the other observed effects were due to direct interactions of the pups, or indirectly a consequence of differences in maternal behavior with different types of litters is not known. It appears there may be a neural mechanism affected by the sex of the individual that reinforces the complete activation or depression of COX activity when a WT sibling of the same sex is present. This can be taken to mean that a WT sibling of the same sex induces a physiological or neurological response that alters nuclei and WB activity. Since KO and opposite sex siblings do not induce this response, there is likely some differentiation by the individual (or possibly mother) of what is a normal sibling.

Most social information is gained through olfaction in rodents and, after transduction of chemosensory signals, transmitted through well-defined neural circuits (Blaustein, 2008; De Vries and Simerly, 2002; Hull and Rodriguez-Manzo, 2008; Newman, 1999). Prominent nuclei in this system are the medial amygdala (including the MEAA, MeAPD, and MeAPV), which project to the bed nucleus of the stria terminalis (including the BST, BSTMA, and BSTMPM) and, in turn, to the medial preoptic area and anterior hypothalamus. In the present study COX activity in the MeAPD, MeAPV, BSTMA, and AHA, was

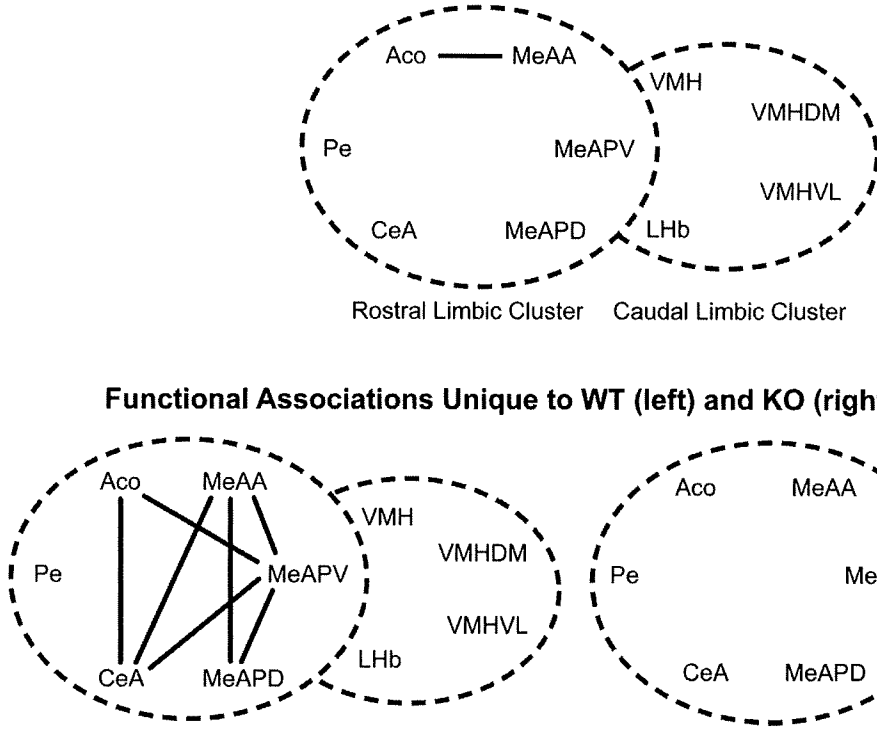

FIGURE 10 | Effect of the individual's genotype on functional connectivity in female mice independent of the sibling's genotype or sex. The top panel represents the shared correlations after jackknifing common to WT and KO females. The bottom panel represents the shared correlations connections unique to WT (left) or KO (right) females. Connections based on Table 2A in Supplementary Material, $\alpha = 0.01$. Each series of nuclei encircled by a dotted line represents a limbic cluster. The left circle in the foreground is the mRLC and the right circle in the background is the mCLC between each genotype.

significantly lower in males raised with WT brothers compared to males raised with KO brothers. Further, males raised with WT sisters had higher COX activity in the MeAPV and the VMHVL compared to males raised with WT brothers. The functional associations of metabolic activity in the network of nuclei was also significantly modified by the litter composition. The differences in limbic landscapes of male and female mice of different genotype and sibling type captured in **Figures 8, 9, 12, and 13** illustrate the effects of Sex versus Genotype in both WT and KO male and female mice. Note that the effect of Sex (male versus female) is substantially different among WT and KO mice, just as the effect of Sibling Genotype is different between male and female mice. It is of interest that ♂KO and ♀WT share similar means, although the female is not statistically different from male WT (possibly due to decreased sample size) for all nuclei except the VMHVL, suggesting this nucleus may be affected by female siblings preferentially. Further, the behavioral differences observed are likely a function of some combination of altered WB, nuclei, or network activity. For example, males raised with ♂WT siblings behave differently than do males raised with ♂KO siblings. This may result from lowered WB activity or from one or more specific nuclei being significantly lowered in activity. The AHA is significantly lowered in activity for males raised with ♂WT siblings and also appears to be strongly correlated with several other nuclei in the RLC and not at all in males raised with ♂KO siblings.

Principal Component Analysis appeared to successfully determine portions of underlying networks of functionally coupled nuclei. Applying the jackknifing procedure to these networks can be thought of as looking for strong correlations in nuclei preselected for probable strong correlations (Sakata et al., 2000). In support of what appears to be an arbitrary choice of significance in the use of PCs, the resulting functional connections in the cluster were not completely interconnected (which would imply too high of a selection criteria for the PC coefficient), nor too large to begin interpretation (too low of a selection criteria for the coefficient), nor was there an excessively large list of nuclei to even begin interpretation and comparison (also suggesting too low of a selection criteria). Using PCA revealed males to have a network of functional associations more conserved between genotypes than females, possibly due to a smaller nuclei sample in females. The observation that the first and second PCs shared similar coefficients between Genotypes led to the reduction of the 20 nuclei into two independent clusters, the RLC and the CLC. These clusters shared two nuclei throughout jackknifing, the VMH and the PaN. While the VMH met the selection criterion in one of the PCs for both clusters, the PaN did not meet selection criteria in the mCLC. Thus, the PaN was used to determine if lower selection criterion for the PCs would yield more meaningful results. This comparison showed only four significant functional correlations between the PaN and some other nucleus

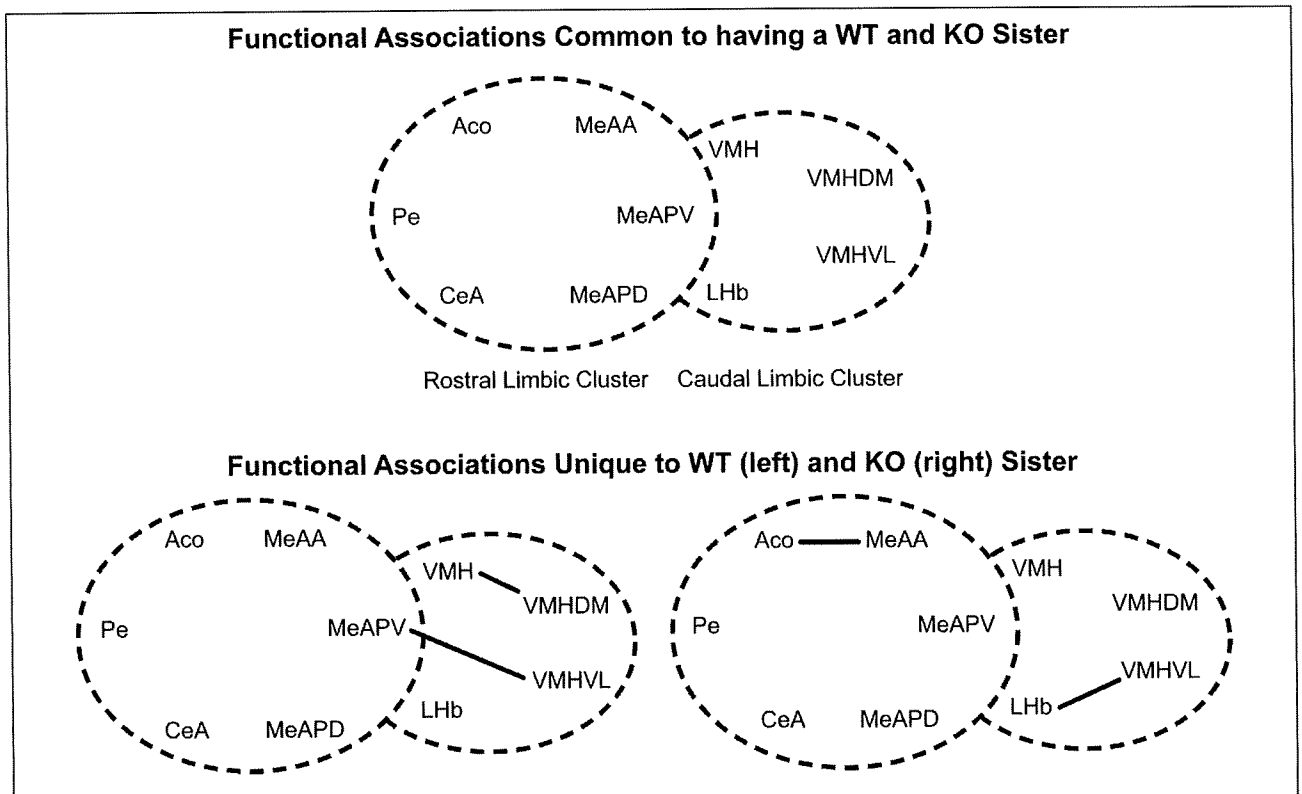

FIGURE 11 | Effect of sibling genotype on functional connectivity in female mice raised in litters consisting of WT or KO sisters independent of the genotype of the individual. Top panel indicates functional connections common to females regardless of genotype of littermates. Bottom panel indicates functional connections unique to females having WT sisters (left) or KO sisters (right). Connections based on Table 2A in Supplementary Material, $\alpha = 0.01$. Each series of nuclei encircled by a dotted line represents a limbic cluster. The left circle in the foreground is the mRLC and the right circle in the background is the mCLC.

in the CLC across all groups; three of these connections occurring between the PaN and VMH in the RLC. On the other hand, the VMH had nine connections in the CLC across all groups, three of which were shared with the PaN. There were six unique connections with the VMH in the CLC, compared to one in the PaN, suggesting that the VMH was actually functionally connected in both clusters of nuclei, whereas the PaN was likely just part of the RLC, the cluster in which it met selection criterion. The one connection of the PaN in the CLC with the MeAPV (**Figure 7**) is likely a functional circuit involving the VMH as this nucleus connects with both the PaN and the MeAPV. Consequently, the functional connection between the MeAPV and PaN could be mediated by the VMH.

The selection of clusters according to the results of PCA suggest a physically relevant cluster of nuclei, as the clusters were relatively conserved between Genotypes and the nuclei were placed in clusters without preconceptions about interactions between nuclei (excluding the placement of the PaN in the CLC). The nuclei considered in the RLC are associated with very different functions, albeit all of which have been related to mediating aggressive and sexual behaviors. The nuclei constituting the CLC have been implicated in sociosexual behaviors as well as feeding behavior. It is interesting that in certain instances anatomically associated nuclei were in different clusters. Nuclei that are very close to one another were usually quite similar in metabolic activity, so functional connectivity often follows. However, subnuclei in the medial amygdala – containing the MeAA, MeAPD, and MeAPV – and in the ventromedial hypothalamus – containing the VMH, VMHVL, and VMHDM, did not show strong correlations to one another. The MeAA was placed in the RLC and the MeAPD and MeAPV were placed in the CLC with little support for the MeAA to be placed with the posterior medial amygdaloid nuclei. This segregation of anterior and posterior medial amygdalar nuclei agrees with Newman (1999) who argued that the "medial extended amygdala" is divided into an anterior functional circuit and a posterior functional circuit. The VMH, on the other hand, divided independent of the VMHDM and VMHDL into the RLC, but also divided with these nuclei into the CLC. This suggests that two functional circuits, one which shares the anterior nuclei, that is the MeAA and VMH, and another posterior functional circuit that shares functional connectivity between the VMH, VMHVL, VMHDM, MeAPD, MeAPV. In support of the expected functional connectivity of proximal anatomic nuclei, the MeAPV connecting with the MeAPD, and the VMHVL connecting with the VMHDM, showed some of the most consistent functional connections among all groups. However, in the CLC the VMH showed functional connectivity with the MeAPV more frequently than either of the more caudal VMH subnuclei across groups. The reason for this functional dissociation of the VMH from the posterior VMH nuclei is not known, but may be related to the anterior–posterior division of the medial amygdala and ventromedial hypothalamus subnuclei discussed above.

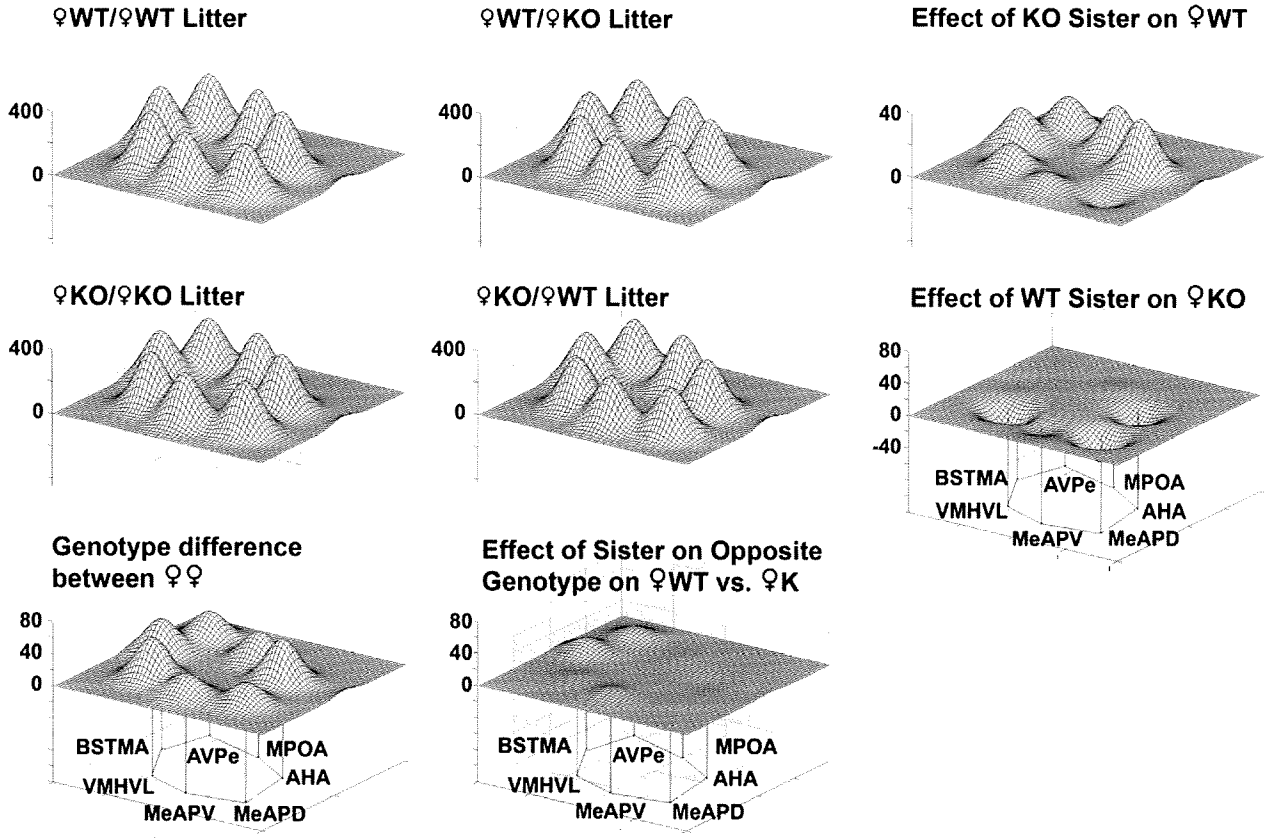

FIGURE 12 | Effect of genotype of sisters of on metabolic activity in a social behavior network in female wildtype (WT) and estrogen receptor a knockout (KO) mice. Groups are presented according to the type of sibling with which the WT (top row) or KO female (middle row) was raised with ♀KO or ♀WT sisters, respectively. The limbic landscape map on the upper right represents the difference in ♀WT raised with ♀KO sister. Below that is the complement, that is, the effect of a WT sister on the metabolic activity in ♀KO females. The bottom row indicates genotype differences between ♀WT and ♀KO females raised in same-sex, same-genotype litters. Right column is the effect of having a sister having the opposite genotype. Other details as in **Figure 8**.

The significant correlation coefficients (**Table 3B**) can be thought of as functional connections or associations, meaning that these correlations associate the COX activity in one nucleus with the COX activity in another nucleus, demonstrating that the two nuclei are functionally coupled in the animal. These associations do not imply directionality of an effect, or, even in the case of a demonstrated neuroanatomical connection, that the correlation is caused by direct activation of one nucleus upon another (Gonzalez-Lima and McIntosh, 1995). The correlation coefficients can only show how well the COX activity in one nucleus can predict the COX activity in another nucleus. The physiological meaning of this correlation, and, consequently, how the jackknifed correlations are to be interpreted depends on what a functional connection between nuclei reveals about the biological system. When a significant correlation occurs between two nuclei using COX activity, it may be understood that increased or decreased activity in one nucleus associates with the increased or decreased activity of the other nucleus. As COX activity shows the capacity of a cell to perform under metabolic demand, functional connectivity may be thought of as showing that the maximal metabolic capability of two nuclei to perform is associated (Sakata et al., 2005).

At the cellular level, this association is given greater meaning. Increased demand in energy by the cell has been shown to activate the bigenomic machinery responsible for the production of COX (Scarpulla, 2006), and, in the neuron, metabolic demand is mostly attributed to maintaining the proper membrane potential using the Na^+/K^+ ATPase (Wong-Riley, 1989). Since the depolarized neuron has a greater demand repolarize its membrane, it follows that energy consumption and the amount of COX present will depend upon the amount of tonic activation of a neuron due to excitatory signals from other neurons (Wong-Riley, 1989). Consequently, abundant COX activity within a given nucleus represents consistent activation by excitatory signals. Thus, functional connectivity reflects regions whose activity is correlated to some degree. Strong functional connections (high correlations ~0.8–0.9) can even be thought of as functional circuits due to the near complete coupling of activity between the two nuclei. However, such functional connections cannot be thought of as anatomical circuits due to the possibility of an unmeasured nuclei being involved. In fact, in the case of measurements made in the limbic brain, such as this study, it is almost impossible to consider every nucleus involved in a hypothesized circuit. Functional connections found between nuclei

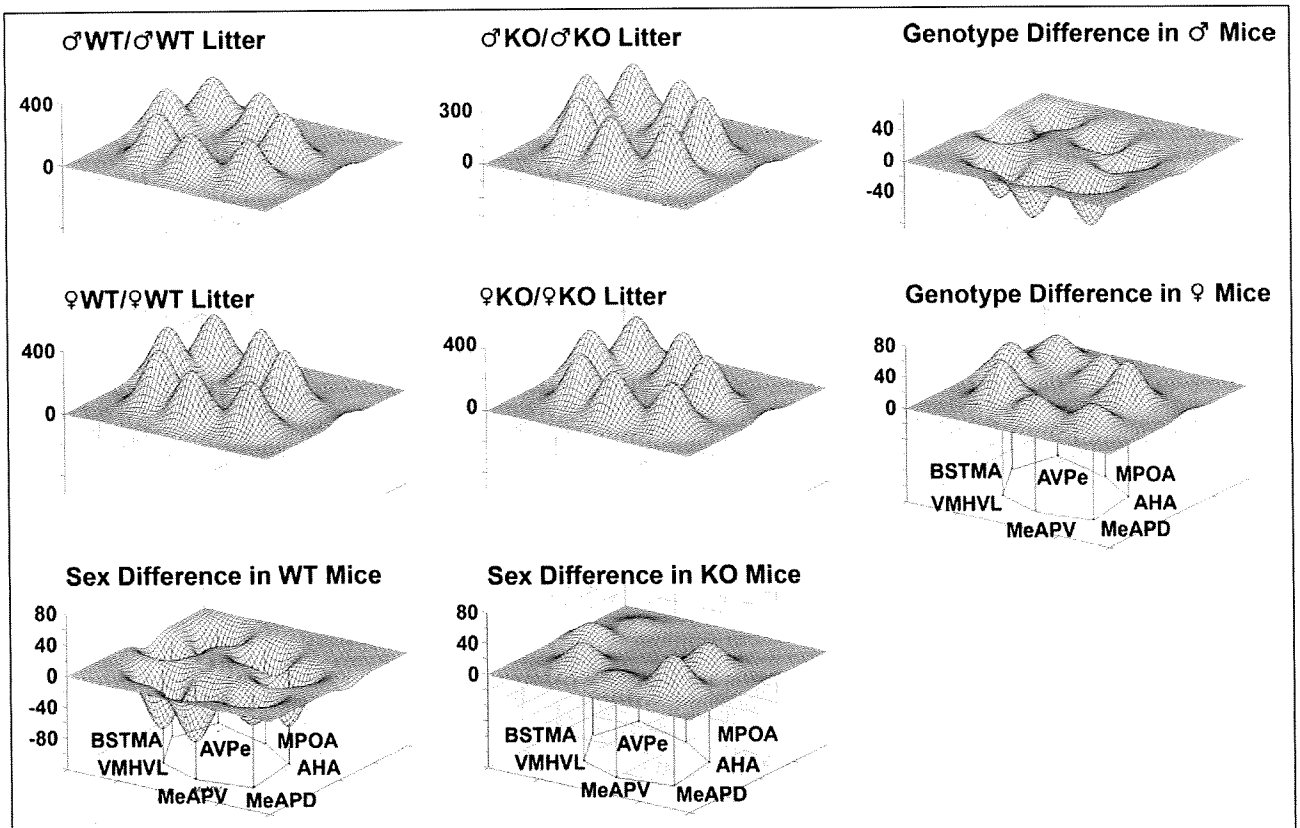

FIGURE 13 | Sex versus genotype differences in wildtype (WT) mice and mice having a dysfunctional estrogen receptor a (KO) mice. Groups consisted of same-sex, same-genotype litters (♂WT/♂WT; ♂KO/♂KO; ♀WT/♀WT; ♀KO/♀KO). The limbic landscape map on the upper right represents the genotype difference in male mice and below that is the genotype difference in female mice. The bottom row indicates sex differences between male and female WT mice and to the right is the sex difference in KO mice. Other details as in **Figure 8**.

in this study should then be considered as possible components of circuits.

Within each cluster certain functional connections are conserved independent of litter composition (i.e. Genotype or Sibling Type), suggesting that these functional connections are maintained independent of treatment. Both KO or WT males shared certain functional connections: MeAA with ACo, CeA, PaN, and VMH, and the ACo with CeA in the mRLC, and MeAPD with MeAPV and the VMHDM with VMHVL in the mCLC. Some of these functional connections are reasonable to expect due to the close proximity of each nuclei or subnuclei such as the functional connections between the MeAPD with MeAPV, and the VMHDM with VMHVL, or the slightly more distant functional connections between the MeAA, ACo and CeA. However the relatively distant functional connections between the MeAA and the PaN and VMH suggests that the MeAA is acting in a conserved functional connection or circuit. In comparing the effect of male Sibling Type on functional connectivity the following relationships maintained significance at $\alpha = 0.01$ level: the CeA with ACo and MeAA, MeAA with VMH, MeAPD with MeAPV, and VMHVL with VMHDM. This differs from the conserved connection between Genotypes in the loss of a functional connection of the MeAA with the ACo, CeA, and PaN. The above comparisons of functional connections are given at $\alpha = 0.01$, but there are some differences in correlations between comparable groups for Genotype or Sibling Type that are maintained at $\alpha = 0.05$. However, many other functional connections become significant for both Genotype and Sibling Type at $\alpha = 0.05$. Those new functional connections that are significant across treatments are the AHA with the PaN, the MeAA with the PaN, and the MeAPV with the VMHVL.

Large differences between groups in functional connectivity are possible indicators of connections and circuits that may contribute to differing behavioral phenotypes. In comparing Genotype, it appears that WT males have many more significant functional connections that appear in the mRLC in comparison to KO males, who have relatively few unique functional connections in this cluster (**Figure 6**). WT males show several strong connections that are not present in KO males (e.g., AHA with CeA, VMH, MeA, and cMPOA; cMPOA with CeA and AHA). Many of these nuclei are known to be involved in male sexual behavior and social interactions. The AVPe only shows one functional connection in the WT males (AVPe with VMH) and this connection is likely due to a large axonal connection between the AVPe and the BST that is known to be dependent upon estrogen for its formation. In KO animals, a unique functional connection between the VMH and the MeAPD and MeAPV was seen that had no counterpart in WT males.

The males having either WT or KO brothers shared certain functional associations: the ACo with the CeA, the CeA with the MeA, and the MeA with the VMH in the RLC and between the MeAPD and the MeAPV and between the VMHVL and the VMHDM (**Figure 7**). Males having WT brothers had many more functional associations than if they had KO brothers, although this may have been due to the number of males considered in each group.

Behavioral neuroscience has been characterized by a candidate nuclei/gene approach that is a gross oversimplification of what occurs in the brain during behavior. The fact that nuclei (or genes) do not operate in isolation but in networks may seem obvious, but this is not reflected in how most scientists write or communicate. Newman (1999) argued that an extended network of interconnected brain nuclei, including many of the nuclei measured in this study, functions in most basic social behaviors in rodents and, further, that this network is modulated by gonadal sex steroid receptors to function differently under different conditions. Another recent study taking alternative approaches has also emphasized a network approach to describe neural systems involved in responding to subordinate conspecific intruders (Motta et al., 2009). The present results indicate that an individual's experience in the litter influences its behavior as an adult by influencing the metabolic activity in integrated limbic circuits. This and previous research indicate that such experiences also shape how different individual respond to events later in life. This work is important for several reasons. First, it demonstrates a profound effect of the Sex and Genotype ratio of the litter on brain and behavior of genetically modified mice. Even in highly inbred rodents all litters are different, varying not only in the sex ratio and parity of the mother, but in the case of genetically modified animals, the genotype of the individual members. Such animals have been a mainstay in the area of molecular behavioral neuroscience, yet virtually every study to date has ignored the litter as a source of variation. By deconstructing the two variables (Sex and Genotype) that define the litter we show how the interplay of these factors shapes the neural substrates of behavior in the commonly used estrogen receptor KO mouse model. Second, we demonstrate how complex behavioral traits depend upon networks of nuclei whose functional relationships are altered fundamentally as a result of the litter environment in which the individual is raised. Third, we show how the potential for behavioral differences is based on the abundance of COX in specific brain nuclei and how the genotype (WT or KO) of brother and sister littermates modifies the amount of COX in limbic nuclei identified. Fourth, the work addresses key issues in how experiments in this very large field should be designed to yield a deeper understanding of how neural systems are organized early in life, particularly if the scientist's goal is to reveal more about how the individual is formed and functions. We argue that most studies today focus at the level of the control and consequences of gene action and hence miss the larger picture. Finally, this work touches on fundamental concepts of development of the neural substrates of behavior, such as how functional systems can be re-organized depending upon the composition of the litter in which the individual develops. Most important in this genocentric age, the ever-increasing use of genetically modified animals in behavioral neuroscience research makes in imperative that practitioners be aware of this important formative element.

ACKNOWLEDGMENTS

This research supported by MH 068273 (DC). We thank Nicolle Mathews, Samantha Luk, and Laura Murphy, Tomoko Soga, and Kazuya Tomihara for research assistance, Gwen Gage and Vicky Huang for help in the graphics, and Kyoko Matsui and Vivian Chin for behavioral data analysis and Lauren Ancel Myers for her helpful suggestions about statistical methods.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/behavioralneuroscience/paper/10.3389/neuro.08/012.2009/

REFERENCES

Bateson, P. (2005). The return of the whole organism. *J. Biosci.* 30, 31–39.

Blaustein, J. D. (2008). Feminine reproductive behavior and physiology in rodents: integration of hormonal, behavioral, and environmental influences. In Hormones Brain and Behavior, Vol. 1, D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, and R. T. Rubin, eds (San Diego, CA, Academic Press).

Catell, R. B. (1966). The SCREE test for the number of factors. *Multivariate Behav. Res.* 1, 245–276.

Crews, D. (1999). Sexuality: the environmental organization of phenotypic plasticity. In Reproduction in Context, K. Wallen and J. Schneider, eds (Cambridge, MIT Press), pp. 473–499.

Crews, D. (2008). Epigenetics and its implications for behavioral neuroendocrinology. *Front. Neuroendocrinol.* 29, 344–357.

Crews, D., Fuller, T., Mirasol, E. G., Pfaff, D. W., and Ogawa, S. (2004). Postnatal environment affects behavior of adult transgenic mice. *Exp. Biol. Med.* 229, 935–939.

Crews, D., Gore, A. C., Hsu, T., Dangleben, N. L., Spinetta, M., Schallert, T., Anway, M. D., and Skinner, M. K. (2007). Transgenerational epigenetic imprints and mate preference. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5942–5946.

Crews, D., and Groothuis, A. G. G. (2005). Tinbergen's fourth question: ontogeny. *Anim. Biol.* 55, 343–370.

Crews, D., Lou, W., Fleming, A., and Ogawa, S. (2006). From gene networks underlying sex determination and gonadal differentiation to the development of neural networks regulating sociosexual behavior. *Brain Res.* 1126, 109–121.

Crews, D., and McLachlan, J. A. (2006). Epigenetics, evolution, endocrine disruptors, health and disease. *Endocrinology* 147(Suppl.), S4–S10.

De Vries, G. J., and Simerly, R. B. (2002). Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In Hormones Brain and Behavior, Vol. 4, D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, and R. T. Rubin, eds (San Diego, CA, Academic Press), pp. 137–191.

Ecker, C., Reynaud, E., Williams, S. C., and Brammer, M. J. (2007). Detecting functional modes in large-scale cortical networks with functional magnetic resonance imaging: a principal component analysis of the human visual system. *Hum. Brain Mapp.* 28, 817–834.

Fleming, A. S., Kraemer, G. W., Gonzalez, A., Lovic, V., Shah, A., Rees, S., and Melo, A. (2002). Mothering begets mothering: the transmission of behavior and its neurobiology across generations *Pharmacol. Biochem. Behav.* 73, 61–75.

Gonzalez-Lima, F., and Cada, A. (1998). Quantitative histochemistry of cytochrome oxidase activity. In Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease, F. Gonzalez-Lima, ed. (New York, NY, Plenum), pp. 55–90.

Gonzalez-Lima, F., and McIntosh, A. R. (1995). Analysis of neural network interactions related to associative learning using structural equation modeling. *Math. Comput. Simul.* 40, 115–140.

Gottlieb, G. (2002). Individual Development and Evolution: The Genesis of Novel Behavior. Mahwah, NJ, Lawrence Erlbaum Associates.

Hull, E. M., and Rodriguez-Manzo, G. (2008). Male sexual behavior. In In Hormones Brain and Behavior, Vol. 1, D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, and R. T. Rubin, eds (San Diego, CA, Academic Press).

Jablonka, E., and Lamb, M. J. (1995). Epigenetic Inheritance and Evolution. Oxford, Oxford University Press.

Jones, D., Gonzalez-Lima, F., Crews, D., Galef, B. G., and Clark, M. M. (1997). Effects of intrauterine position on the metabolic capacity of the hypothalamus of female gerbils: a cytochrome oxidase study. *Physiol. Behav.* 61, 513–519.

Lewontin, R. C. (2000). The Triple Helix: Gene, Organism and Environment. Cambridge, Harvard University Press.

Loehlin, J. C. (2004). Latent Variable Models: An Introduction to Factor, Path, and Structural Equation Analysis. Kentucky, Lawrence Erlbaum Associates, Inc.

Meaney, M. J. (2001). Maternal care, gene expression and the transmission of individual differences in stress reactivity across generations. *Annu. Rev. Neurosci.* 24, 161–192.

Moore, C. L. (1995). Maternal contributions to mammalian reproductive development and divergence of males and females. In Advances in the Study of Behavior, P. J. P. Slater, J. S. Rosenblatt, C. T. Snowdon, and M. Milinski, eds (New York, NY, Academic Press), pp. 47–118.

Moore, C. L., Wong, L., Daum, M. C., and Leclair, O. U. (1997). Mother-infant interactions in two strains of rats: implications for dissociating mechanism and function of a maternal pattern. *Dev. Psychobiol.* 30, 301–312.

Motta, S. C., Goto, M., Gouveia, F. V., Baldo, M. V., Canteras, N. S., and Swanson, L. W. (2009). Dissecting the brain's fear system reveals the hypothalamus is critical for responding in subordinate conspecific intruders. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4870–4875.

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior: a node in the mammalian social behavior network. *Ann. N. Y. Acad. Sci.* 877, 242–257.

Ogawa, S., Chan, J., Gustafsson, J. A., Korach, K. S., and Pfaff, D. W. (2003). Estrogen increases locomotor activity in mice through estrogen receptor alpha: specificity for the type of activity. *Endocrinology* 144, 230–239.

Ogawa, S., Eng, T., Taylor, J., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1998b). Roles of estrogen receptor: a gene expression in reproduction-related behaviors in female mice. *Endocrinology* 139, 5070–5081.

Ogawa. S., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1996). Reversal of sex roles in genetic female mice with disruption of estrogen receptor gene. *Neuroendocrinology* 64, 467–470.

Ogawa S., Lubahn, D. B., Korach, K. S., Pfaff, D. W. (1997). Behavioral effects of estrogen receptor gene disruption in male mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 1476–1481.

Ogawa, S., Luk, S. Murphy, L., Matthews, D., Pfaff, D. W., Tomihara, K., Soga, T., and Crews, D. (2005). Effects of the litter composition during preweaning period on the development of anxiety-related behaviors in mice. *Soc. Neurosci. Abs.* 31, 892.14.

Ogawa, S., Washburn, T. F., Taylor, J., Lubahn, D. B., Korach, K. S., and Pfaff, D. W. (1998a). Modifications of testosterone-dependent behaviors by estrogen receptor: a gene disruption in male mice. *Endocrinology* 139, 5058–5069.

Paxinos, G., and Franklin, K. (2001). The Mouse Brain Atlas in Stereotaxic Coordinate, 2nd edn. San Diego, CA, Academic Press.

Sakata, J. T., Coomber, P., Gonzalez-Lima, F., and Crews, D. (2000). Functional connectivity among limbic brain areas: Differential effects of incubation temperature and gonadal sex in the leopard gecko, *Eublepharis macularius. Brain Behav. Evol.* 55, 139–151.

Sakata, J. T., Crews, D., and Gonzalez-Lima, F. (2005). Behavioral correlates of differences in neural metabolic activity. *Brain Res. Rev.* 48, 1–15.

Sakata, J. T., Gonzalez-Lima, F., Gupta, A., and Crews, D. (2001). Animal models of experiential effects on neural metabolism: Plasticity in limbic circuits. In Neuroplasticity, Development and Steroid Hormone Action, R. Handa, S. Hayashi, E. Terasawa, and M. Kawata, eds (Boca Raton, FL, CRC Press), pp. 257–272.

Sakata, J. T., Gonzalez-Lima, F., Gupta, A., and Crews, D. (2002). Repeated interactions with females elevate metabolic capacity in the limbic system of male rats. *Brain Res.* 936, 27–37.

Scarpulla, R. C. (2006). Nuclear control of respiratory gene expression in mammalian cells. *J. Cell Biochem.* 97, 673–683.

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature* 150, 563–565.

Waddington, C. H. (1953). Genetic assimilation of an acquired character. *Evolution* 7, 118–126.

West-Eberhard, M. J. (2003). Developmental Plasticity and Evolution. New York, NY, Oxford University Press.

Wong-Riley, M. T. (1989). Cytochrome Oxidase: an endogenous metabolic marker for neuronal activity. *Trends Neurosci.* 12, 94–101.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 May 2009; paper pending published: 02 July 2009; accepted: 17 July 2009; published online: 14 August 2009.

Citation: Crews D, Rushworth D, Gonzalez-Lima F and Ogawa S (2009) Litter environment affects behavior and brain metabolic activity of adult knockout mice. Front. Behav. Neurosci. 3:12. doi:10.3389/neuro.08.012.2009