Depressive Behavior and Activation of the Orexin/Hypocretin System

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The orexin/hypocretin peptide signaling system plays a neuromodulatory role in motivation and stress; two critical components of depression. Although work has been done to identify links between orexin and depression, few specific neuroanatomical associations have been made. These studies have not investigated the relationship between orexin and orexin receptor expression in specific brain regions associated with this disorder. To address this, we examined immobility during the forced swim test (FST) in mice, a commonly used measure of depressive behavior. We analyzed the variation in FST immobility with the distribution of orexin and its receptor mRNA. We found that animals that exhibited more robust depressive behavior had greater or lesser orexin system expression that depended on the limbic brain region analyzed. In the hippocampus there was a negative correlation between orexin expression and FST immobility. Animals that displayed relatively more depressive behavior had lower hippocampal expression of Orexin A (OrxA). In the amygdala, there was a curvilinear relationship between OrxA and FST performance. In addition there was a positive correlation with amygdalar Type I orexin receptor (Orx1) mRNA and depressive behavior. Despite the differences in limbic orexin expression, there was no correlation between immobility and hypothalamic orexin neuron activation as measured by c-Fos. Overall, more severe depressive behavior was associated with reduced hippocampal orexin expression, contrasted with increased orexin plus Orx1 receptor mRNA expression in the amygdala. This divergent pattern between the hippocampus and amygdala mirrors a neurobiological theme seen in depression resulting from reduced hippocampal, but increased amygdalar, size and function.

Keywords: depression, hypocretin, orexin, stress, forced swim test

Since orexin (hypocretin) was discovered in 1998 the peptide has been implicated in a number of processes that are also involved with depression. Specifically, the disease affects two main categories of behavior: motivation and stress (Harris & Aston-Jones, 2006). Orexin is involved with these behaviors via hypothalamic orexin projections that innervate the ventral tegmental area (VTA), which is associated with motivation and arousal (Fadel & Deuthe, 2002; Koob & Le Moal, 2001). The corticotropin releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus (PVN) which make up the proximal end of the stress response are also integrated with the orexin system (Vale, Spiess, Rivier, & Rivier, 1981; Winsky-Sommerer, Boutrel, & de Lecea, 2005). In
addition to the VTA, orexin fibers innervate and stimulate other monoamine centers that are associated with depression, such as raphé (Brown, Sergeeva, Eriksson, & Haas, 2001; Wang et al., 2005), and locus ceruleus (LC; Peyron et al., 1998; Walling, Nutt, Lalies, & Harley, 2004). Orexin perikarya are located exclusively in the hypothalamus, but orexinergic projections and receptors are broadly distributed in the brain (Chen, Dun, Kwok, Dun, & Chang, 1999; Trivedi, Yu, MacNeil, Van der Ploeg, & Guan, 1998). Much of the work examining causative relationships between orexin and depression involves systemic manipulations of the orexin system. However, some of these experiments have contradictory results for the orexin system; some prodepressive (Nollet et al., 2011) and others antidepressive (Ito et al., 2008).

Stress activates (c-Fos) hypothalamic orexin neurons (Nollet et al., 2011; Sakamoto, Yamada, & Ueta, 2004). The relationship between orexin and stress is noteworthy, given that chronic stress is a reliable predictor of depression, and may be necessary for establishment of the disorder (Duric & Duman, 2012; Hammen, Kim, Eberhart, & Brennan, 2009). Depression produces distinctive neuroanatomical changes, such as reduced hippocampal and prefrontal cortices or enlarged amygdalae, which may be reversed with antidepressants (Krishnan & Nestler, 2010; MacQueen & Frodl, 2010; Pittenger & Duman, 2008). The hippocampus and prefrontal cortex are brain areas that inhibit stress responses and have been shown to limit depressive behavior (MacQueen & Frodl, 2010). In contrast, the amygdala, BNST, and subgenual cortex promote anxiety and/or stress responsiveness and are associated with depressive phenotypes (Hamani et al., 2011; Lee, Fitz, Johnson, & Shekhar, 2008; Slattery, Neumann, & Cryan, 2011). All of the regions involved in depression are innervated by the orexin system (Peyron et al., 1998). Although orexin A and B (OrxA, OrxB) are similar in structure, OrxA has a disulfide bridge and binds to the orexin 1 (Orx1) receptor with greater affinity (de Lecea et al., 1998; Sakurai et al., 1998). The orexin 2 (Orx2) receptor has equal affinity to both OrxA and OrxB. In line with stress-induced activation of orexin neurons, a chemically induced depressive state leads to increased amounts of both OrxA and OrxB in adult rats (Feng, Vurbic, Wu, Hu, & Strohl, 2008).

In orexin receptor knockout mice, Orx2 receptors were demonstrated to have antidepressive properties, whereas Orx1 receptors were prodepressive (Scott et al., 2011). Unpredictable chronic mild stress, a putative model of depression, causes little change in Orx1, Orx2, and Orx3, whereas Orx1, Orx2, and Orx3 are increased in the ventral hippocampus, thalamus, and hypothalamus (Nollet et al., 2011). These stress-induced thalamic and hypothalamic Orx2 receptor decreases are reversed in response to the serotonin uptake inhibitor fluoxetine. This suggests that Orx2 receptors play a role in the pathophysiology of depression.

In contrast, the dorsal hippocampus and amygdala in response to a stressful situation (Arendt et al., 2012). We expected the orexin system to show a similar activity pattern to BDNF. Orexin operates through stimulatory pathways and has been shown to increase BDNF expression (Akbari, Motamed, Davoodi, Noorbakhshnia, & Ghanbarian, 2011; Lund et al., 2000; Yamada et al., 2009). Furthermore, we expected animals exhibiting relatively healthier behavior to display increased Orx2 receptor mRNA expression, relative to Orx1, given that the Orx2 receptor knockout mice show a depressive phenotype (Scott et al., 2011).

Method

Subjects

Adult male c57bl/6 mice (Harlan, Indianapolis, IN; n = 29) were housed in pairs on a 12:12 light–dark cycle (lights off at 10am) with food and water provided ad libitum. All testing took place between 10 a.m. and 2 p.m. All animal experiments were executed in a manner that minimized suffering and the number of animals used, in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), and approved by the Institutional Animal Care and Use Committee of the University of South Dakota.

Behavioral Testing

The forced swim test (FST) consisted of 2 days of testing. On the first day animals (n = 25) were placed in a clear acrylic 3-L cylinder (12 cm diameter) half filled with 25°C water for 15 minutes and subsequently dried in a heated area of the room for another 15 minutes before being returned to their home cage. On the second day, animals were again placed in the cylinder of water, this time for 5 minutes, and dried before being killed one hour after being taken out of the water. Control animals for cell counting were handled and transported to the behavior suite adjacent to housing quarters but were not subjected to FST (n = 4). All behavioral tests were recorded on video and scored by multiple observers blind to treatment, for total duration of immobility which was defined as the minimal amount of movement required for the animal to stay afloat.

Immunohistochemistry

Animals (n = 12) were sedated with isoflurane and perfused transcardially with ice-cold PBS followed by 4% paraformaldehyde 1 h after the FST was complete. Brains were removed and stored in a 4% paraformaldehyde solution at 4°C for 24 h before being cryoprotected in 30% glycerol/phosphate-buffered saline (PBS) solution. Brains were then frozen on powdered dry ice and stored at −80°C. Coronal brain sections (35 μm) were taken from a cryostat microtome (Leica, CM 1850) and stored at 4°C in PBS with 0.01% NaAzide until analysis. All sections were dual labeled in goat antiorixin A (1:300; Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-Fos (1:20,000; EMD4 Biosciences, Gibbstown, NJ) at 4°C for 24 hours with gentle agitation on a rocker plate. Primary antibody dilutions were done in PBS with 3% normal donkey serum and 0.25% Triton X-100. Sections were then incubated with secondary antibodies that had DyLight fluorophores conjugated to the antibody (DLS49 donkey antigen; D4488 donkey antirabbit; Jackson ImmunoResearch, West Grove, PA) in PBS at 1:200. No amplification steps were used in the fluorescence.

Coronal slices were visualized using a Zeiss LSM 700 microscope at ×10 magnification. Cell counting was done over three slices of the hypothalamus that robustly expressed orexin neurons, using epifluorescent mode with the pinhole wide open to maximize...
acquisition at deeper layers. Stitch functions were used to image the entire hypothalamus. Each image was independently scored first identifying all orexin positive neurons, and then for nuclei expressing c-Fos. All orexin neurons were also categorized as occupying either the lateral (LH) or dorsomedial-perifornical (DMH/PeF) region of the hypothalamus. For densitometric analysis of terminal brain regions, images of mouse stereotactic maps (Paxinos & Franklin, 2004) were overlaid onto the images of the slices (taken from −1.22 to −2.06 mm AP from bregma) to accurately identify regions of interest (hippocampus, amygdala, thalamic paraventricular nucleus, and hypothalamus) on each slice. The scale for luminosity represents the mean luminosity adjusted for area and normalized to another region with low Orx expression. All regions were normalized to luminosity of the internal capsule, which had consistently low levels of orexin fluorescence. Identification of subregions and densitometry were performed with Photoshop software (Adobe, San Jose, CA).

Brain Microdissection and qPCR

Mice (n = 13) were sedated with 3% isoflurane and decapitated with brains frozen in isopentane on dry ice. Dorsal hippocampi and amygdalae were microdissected from frozen coronal sections (300 μm) using a Peltier cold plate (T° = −25°C; Physitemp, Clifton, NJ) using a sterile blunt 18 gauge needle and immediately injected into TRIzol (Life Technologies, Grand Island, NY) for subsequent RNA purification, and quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Purified RNA (10 μl) was used for complementary DNA (cDNA) synthesis in 20 μl reactions using the High Capacity cDNA Archive Kit (Applied Biosystems, Carlsbad, CA) and purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA). The cDNA product (50 ng) was used in the qPCR reactions on a Step One Plus Real-Time PCR System (Applied Biosystems, Carlsbad, CA). Samples were examined using Taq-man Assay On Demand primer/probe sets (Applied Biosystems) for Orx1 (mm 01185766_m1), and Orx2 (mm 01179312_m1). Each sample was run in duplicate and normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (mm 01179312_m1). Each sample was run in duplicate and normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (mm 01179312_m1). Each sample was run in duplicate and normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (mm 01179312_m1).

Statistics

Total duration of immobility was used to compare depressive behavior with Orx subregional expression (Lutter et al., 2008). During qPCR and immunohistochemistry, low cDNA quantity or lost tissue resulted in some samples being omitted from analyses. Comparisons of orexin neuronal activation between groups (FST vs. Control) and comparisons between DMH/PeF and LH orexin cell populations were made using t tests. Correlative effects between immobility and protein immunofluorescence or gene expressions were examined using regression analysis results.  

Hypothalamic Orexin and c-Fos

There was a robust activation of c-Fos in orexin cell bodies one hour after the final FST trial (t_{13} = 2.308, p < .038; Figure 1A, 1B, and 1C). When this population of cells is subdivided (Figure 1A) in relation to the fornix and mammothalamic tract, the DMH/PeF orexin cell population (Johnson et al., 2010, 2012) is significantly activated by the FST compared with controls (t_{13} = 2.475, p < .028; Figure 1C), and relative to the LH orexin cells (t_{20} = 3.533, p < .002). There was no difference in activation between the DMH/PeF and LH neurons in animals that were not exposed to the FST (controls; t_{6} = −0.630, p < .552). It should be noted that though there was not a significant activation of orexin cells by FST compared with controls in the LH, a trend is evident (t_{13} = 2.067, p < .059; Figure 1C). Although there was considerable variation in the depressive behavior (duration of immobility), this variation could not be explained with orexin cell activation in either the DMH/PeF (F_{1,9} = 0.491, p < .501; Figure 1D) or LH (F_{1,9} = 0.377, p < .554; not shown).

Expression to the FST did not alter the number of individual orexin neurons (not considering c-Fos activation) counted per slide of tissue examined in either the DMH/PeF (t_{13} = 0.623, p < .544), or LH (t_{11} = 0.132, p < .209). It also did not affect densitometric analysis of Orxα expression in these same hypothalamic subfields (DMH/PeF, t_{13} = 1.19, p < .26; LH t_{12} = 0.24, p < .81). The FST did not significantly increase the total number of neurons expressing c-Fos alone (DMH/PeF, t_{13} = −1.580, p < .138; LH t_{13} = −1.656, p < .122). Comparison of single c-Fos expression between the two regions did not differ significantly between control animals (t_{6} = −0.115, p < .912), or animals exposed to the FST (t_{20} = −1.111, p < .280). Single orexin cell counts were not significantly correlated with immobility in either the DMH/PeF (F_{1,9} = 0.015, p < .905, r^2 = 0.002), or LH (F_{1,9} = 0.740, p < .412, r^2 = 0.076). Likewise, there is no correlation between immobility and single c-Fos labeling for either the DMH/PeF (F_{1,9} = 0.867, p < .376, r^2 = 0.088), or LH (F_{1,9} = 0.0003, p < .995, r^2 < 0.0001).

Limbic Terminal Field Orexin A & c-Fos

Concentrations of Orxα (as measured by immunofluorescence) in the hippocampus were negatively correlated with the duration of immobility; with parallel relationships in the hippocampal subregions, dentate gyrus (F_{1,6} = 21.019, p < .004; Figure 2A), CA3 (F_{1,6} = 12.348, p < .013; Figure 2B), and CA1 (F_{1,6} = 17.515, p < .006; Figure 2C). This suggests a common overall relationship between hippocampal Orxα and depressive behavior which is reflected in the regression for Orxα versus duration of immobility in the total dorsal hippocampus (F_{1,6} = 20.375, p < .004; Figure 4A and 4B).

This simple linear regression in hippocampus was in contrast to the relationship between immobility and Orxα in the amygdala where a significant U-shaped curvilinear regression (F_{2,8} = 6.774, p < .019; Figure 4C) was the best fit for the data. A similar significant U-shaped curvilinear regression was seen in subregions of the amygdala including the basolateral (BLA; F_{2,8} = 6.521, p < .021; Figure 3A), medial nuclei (MeA; F_{2,8} = 5.748, p < .028; Figure 3B), and central (CeA; F_{2,8} = 7.243, p < .016; Figure 3C).
The distinctive relationships between depressive behavior and OrxA expression in subregions and the entire dorsal hippocampus (F1,6/H11005 20.375, p/H11021 .004; Figure 4A and 4B), and the subregions and the entire amygdala (F2,8/H11005 6.774, p/H11021 .019; Figure 4C) are suggestive of distinctive function. Similarly, immunostaining for c-Fos in amygdala was significantly correlated with duration of immobility, with a positive linear regression (F1,10/H11005 7.60, p/H11021 .02, r^2/H11005 0.432). Surprisingly, no significant correlation (F1,7/H11005 3.18, p/H11021 .118, r^2/H11005 0.313) between c-Fos and immobility was evident in hippocampus. In addition, there was a significant negative curvilinear regression (F2,4/H11005 12.5, p/H11021 .019, r^2/H11005 0.86) between amygdalar OrxA and the activation (c-Fos) of orexin neurons in the DMH/PeF. The relationships with distinctive function are not present in all regions of the brain. For example, no relationship was seen in the thalamic paraventricular nucleus (F1,8/H11005 0.737, p/H11021 .416; Figure 4D).

**Figure 1.** Immuno-labeled orexin neurons in the (A) DMH/PeF and LH of the hypothalamus, where (B) exposure to the FST significantly (p < .05) increased the number of orexin positive soma (gray = OrxA) expressing c-Fos (white = c-Fos), an effect that was more prominent in the DMH/PeF orexin neurons (C). This activation of orexin neurons did not correlate with individual durations of immobility in either the LH (not shown) or (D) DMH/PeF regions (MT = mammotalthalamic tract, F = fornix).

Receptor mRNA Distribution

As noted, all of our results for OrxA in the dorsal hippocampus and amygdala presented similar correlational relationships between the larger brain area and each of its subregions. Because of that similarity, we limited our receptor gene expression analysis to whole dorsal hippocampus and amygdala. In the dorsal hippocampus there were no significant correlations for either Orx1 (F1,10/H11005 0.224, p < .646; Figure 5A) or Orx2 receptor gene transcripts (F1,10/H11005 2.818, p < .124; Figure 5B) with duration of immobility. While there was no significant relationship for Orx2 mRNA with depressive behavior (F1,11/H11005 0.129, p < .726; Figure 5D) in the amygdala, there was a significant positive correlation between duration of immobility and the gene expression of Orx1 receptor mRNA (F1,11/H11005 6.311, p < .029; Figure 5C) in total amygdala.

**Discussion**

Expression of Orexin A (OrxA) in extrahypothalamic regions exhibited distinctive relationships with depressive behavior that was not evident in the hypothalamic perikarya that produce orexin/hypocretin. It is important to note that the relationships described involve only the inherent variability of despair behavior (immobility) in the forced swim test, and not chronic stress-induced depressive behavior as would be seen after social defeat (Lutter et al., 2008). Although hypothalamic dorsomedial-perifornical (DMH/PeF) OrxA/c-Fos activity did reflect the stress of the forced swim test (FST; Figure 1C) it did not correlate with immobility (Figure 1D). This was in contrast to OrxA expression in hippocam-
pus and amygdala, which reflected differences in FST performance (Figures 2–4). Similarly, expression of mRNA for Orx1 receptors in the amygdala correlated with immobility (Figure 5C). The results suggest that the orexin/hypocretin system may play a role in depressive behavior, via activity in specific brain regions functionally associated with depression.

Orexin neuron activation, as measured by c-Fos, has been associated with both stress and depressive behavior (Harris & Aston-Jones, 2006; Lutter et al., 2008). In our study, forced swim preferentially activated orexin neurons in the DMH/PeF relative to the LH (Figure 1B and 1C). It was not the result of general c-Fos activation in the entire region. Others have also observed preferential activation of DMH/PeF orexin cells during stressful events (Harris, Wimmer, & Aston-Jones, 2005; Johnson et al., 2012). The number of orexin neurons in DMH/PeF expressing c-Fos correlates negatively with the degree of depressive behavior after

Figure 2. There were significant negative linear correlations between Orx₅ concentration (as measured by immunofluorescence) in all subregions of the dorsal hippocampus including the (A) Dentrat Gyrus, (B) CA₃, and (C) CA₁, and the duration of immobility(s) of mice in the FST.

Figure 3. There were significant curvilinear correlations between Orx₅ concentration (as measured by immunofluorescence) in all subnuclei of the amygdala including the (A) basolateral nucleus (BLA), (B) medial nucleus (MeA), and (C) central nucleus (CeA) and the duration of immobility(s) of mice in the FST.
chronic social defeat (Lutter et al., 2008). In contrast, our study showed no relationship between depressive behavior and c-Fos activation in orexin neurons (Figure 1D). However, it is important to note that in our study we did not expose individuals to chronic social stress, nor did we use social avoidance to quantify depressive behavior. Although the hypothalamus may play a role in orexinergic modulation of depressive behavior, we hypothesize that this modulation must include extrahypothalamic activation via orexinergic projections. Our results demonstrate not only significant amygdalar OrxA and c-Fos correlations with depressive behavior, but also a significant regression between DMH/PeF activity (c-Fos) of orexin perikarya and amygdalar OrxA content. As such, our intent was to address the contribution of orexin in extrahypothalamic regions associated with depressive behavior.

Depression reduces the hippocampus in both size and function (Holderbach, Clark, Moreau, Bischofberger, & Normann, 2007; McKinnon, Yucel, Nazarov, & MacQueen, 2009) while enhancing hippocampal synaptic plasticity produces an antidepressant like effect (Govindarajan et al., 2006; Vouimba, Munoz, & Diamond, 2006). Activation of orexin receptors stimulates intracellular calcium influx through a number of intracellular signaling cascades that produce long-term potentiation (LTP; Holmqvist et al., 2005; Lund et al., 2000; Pittenger & Duman, 2008). In the dentate gyrus (DG), antagonism of Orx1 receptors reduces LTP (Akbari et al., 2011). Taken together, these data and our own suggest that hippocampal orexin and its receptors may play an important role in depression. Our results indicate a significant correlation between OrxA, but not Orx1 or Orx2 receptors, and depressive behavior in the CA1, CA3, and DG subregions of the hippocampus. Higher amounts of hippocampal OrxA are associated with a healthier behavioral profile (animals exhibiting less immobility; Figures 2 and 4). The results suggest a hippocampus-wide role for orexin in depressive behavior, and that OrxA activity in the hippocampus may have an antidepressant effect.

In the amygdala, both the OrxA ligand and Orx1 receptor transcript were seen at higher levels in animals that exhibited relatively greater immobility/depressive behavior (Figures 4 and 5). These results for OrxA were consistent in basolateral (BLA), medial (MeA), and central (CeA) subnuclei of the amygdala (see Figure 3). The amygdalae of individuals with depression are hyperresponsive (Drevets, 2000) which would correspond to our results given the excitatory effects of orexin. This does not explain the left

![Figure 4. Duration of immobility(s) was (A) negatively correlated with OrxA immunofluorescence in the (B) whole hippocampus, whereas (C) a curvilinear regression explained the relationship between behavior and OrxA in the amygdala and mouse FST immobility. (D) No relationship was seen between the orexin peptide in the thalamic PVN and duration of immobility.](image-url)
leg of the U-shaped fit for Orx, which shows an increase in the ligand for animals with the lowest durations of immobility. This contradiction may be explained with a receptor-by-ligand interaction invoking the law of mass action. Although OrxA is increased in these animals at the extreme lower range of immobility, this may not translate into stronger overall signaling in conjunction with extremely low Orx1 receptor gene expression (assuming that the mRNA transcript reflects protein translation). Our results for amygdala may be consistent with the results of Scott et al., who showed a prodepressive role for the Orx1 in knockout mice (Scott et al., 2011). Given the broad subnuclear involvement in the orexinergic responses to depressive behavior in the amygdala, as with hippocampus, there appears to be an intraamygdalar circuitry that is broadly activated during stress and depression.

The data from our study suggest that the orexinergic system is not simply expressed in all projections areas equally, relative to inherent expression of depressive behavior such as immobility in the FST. The expression of OrxA appears to be functionally opposite in hippocampus and amygdala relative to depressive behavior, especially when considering the putative utility of the Orx1 receptor transcript, and there seems to be no relation with the paraventricular thalamus at all (Figure 4D). The subregions of the hippocampus (DG, CA3, CA1) and the subnuclei of the amygdala (BLA, MeA, CeA) have internally consistent expression, and perhaps function, for OrxA. Future research on the effects of the orexin system and depression should consider specific orexinergic functions for brain regions associated with depression.

As orexin fibers innervate and stimulate monoaminergic perikarya in the VTA, raphe, and LC (Brown et al., 2001; Moorman & Aston-Jones, 2010; Walling et al., 2004; Wang et al., 2005), a role for orexin in depression seems likely because most antidepressants also increase monoaminergic signaling. A number of studies demonstrate that patients suffering from depression or PTSD have reduced OrxA levels in their cerebrospinal fluid (CSF; Brundin, Bjorkqvist, Petersen, & Traskman-Bendz, 2007; Brundin, Bjorkqvist, Traskman-Bendz, & Petersen, 2009; Brundin, Petersen, Bjorkqvist, & Traskman-Bendz, 2007; Johnson et al., 2010; Strawn et al., 2010). With some exceptions (Schmidt et al., 2011) depression seems to be consistently correlated with decreased CSF levels of OrxA (Brundin, Bjorkqvist, et al., 2007).

**Figure 5.** There were no significant relationships in the dorsal hippocampus between (A) Orx1 receptor mRNA or (B) Orx2 receptor transcript and duration of immobility(s). Whereas a (C) significant positive correlation did exist between amygdalar Orx1 receptor transcript and duration of immobility, (D) no correlation was seen between immobility and amygdalar Orx2 receptor mRNA.
This relationship is more complex in anxiety states in the absence of depression. Patients with PTSD have reduced levels of CSF OrxA (Strawn et al., 2010), whereas those suffering from panic disorder have increased CSF OrxA (Johnson et al., 2010). Furthermore, i.c.v administration of OrxA in rats produces anxiety in the light–dark exploration test (Suzuki, Beuckmann, Shikata, Ogura, & Sawai, 2005). Greater depressive behavior in our study was correlated with decreased OrxA in the hippocampus (Figure 4A) and increased OrxA in the amygdala (Figure 4C). It is evident that understanding specific regional orexinergic activity is necessary to explain the relationship of orexin with depression and/or anxiety.

Conclusions

An important relationship between orexin/hypocretin and depression is suggested by the substantial overlap between the systems affected by the disorder and those modulated by the orexin system. These correlations are corroborated by this study. We demonstrate that inherent variability in depressive behavior (such as immobility during forced swim) is correlated with differences in orexin system projections to specific subregions of the hippocampus and amygdala. Despite slightly elevated levels of amygdalar OrxA associated with very low immobility, the functional relationship was clearly positive. Dramatically increased OrxA peptide concentrations in the basolateral, medial, and central nuclei were measured in conjunction with elevated amygdalar Orx1 receptor mRNA. This appears to represent a functional mechanism for orexinergic activation of the amygdalar nuclei, perhaps in association with fearful and anxious states. This effect in the amygdala appears to represent more than just simple receptor feedback kinetics where increasing amounts of endogenous OrxA reduces the transcript of the receptors, and could possibly be a method for orexin to preferentially activate the amygdala through a positive feedback mechanism (i.e., more ligand yields more receptor). In contrast, individual mice with relatively greater inherent depressive behavior have less OrxA in the DG, CA3, and CA1 subregions of the hippocampus. Depression restructures the brain in both size and function (Drevets, 2000; Lakshminarasimhan & Chattarji, 2012; Pittenger & Duman, 2008), but perhaps endogenous systems (such as orexin) modify specific brain regions differentially. Although further experiments would have to be carried out to confirm that these areas have altered orexin signaling, the differences measured in orexinergic expression in the hippocampus and amygdala are in line with other work that points to opposing effects between these two areas (Arendt et al., 2012; Lakshminarasimhan & Chattarji, 2012). This work elucidates some possible mechanisms for orexin to alter plasticity in these specific brain regions associated with depression. This raises the possibility that manipulations of the orexin system could act as novel treatments for depression.

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