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THE SOCIAL DEFEAT ANIMAL MODEL OF DEPRESSION SHOWS DIMINISHED LEVELS OF OREXIN IN MESOCORTICAL REGIONS OF THE DOPAMINE SYSTEM, AND OF DYNORPHIN AND OREXIN IN THE HYPOTHALAMUS

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Abstract—Anhedonia is a core symptom of clinical depression. Two brain neuropeptides that have been implicated in anhedonia symptomology in preclinical depression models are dynorphin and orexin; which are concentrated along lateral hypothalamic dopamine reward pathways. These affect regulating neuropeptides modulate each other's function, implicating an interactive dysfunction between them in anhedonia symptomology. But whether their influences are modified or imbalanced within the hypothalamus or dopamine system in anhedonic preclinical depression models is not yet clear. We used radioimmunoassay to determine this in the rat social defeat model of depression; at a time that anhedonic sexual disinterest was expressed. In tissue samples of the medial prefrontal cortex (mPFC), ventral tegmental area (VTA) and nucleus accumbens, basal dynorphin levels were similar to normal animals. But orexin was reduced in the VTA and mPFC. Also, dynorphin and orexin were both diminished in the hypothalamus which is noteworthy since nearly all hypothalamic orexin cells co-express dynorphin. These findings suggest that orexin and dynorphin function may be imbalanced between the hypothalamus and mesocortical dopaminergic brain regions in depression.

Key words: orexin, dynorphin, hypothalamus, mPFC, VTA, anhedonia.

INTRODUCTION

A reduced ability to experience pleasure, termed anhedonia, is a hallmark symptom of depression and is expressed in rodent models of the illness. Various studies suggest that a dynorphin and orexin interactive dysfunction between the hypothalamus and the dopamine reward system might exist in depression; and perhaps cause anhedonia symptoms. Dynorphin and orexin modulate each other's function (Eriksson et al., 2004; Li and van den Pol, 2006) as well as brain reward mechanisms; though dynorphin typically inhibits and orexin stimulates neural activity (Shippenberg, 2009; Aston-Jones et al., 2010). Dynorphin is also co-expressed in nearly all orexin neurons, which are exclusively located in dorsomedial and lateral regions of the hypothalamus (Chou et al., 2001). Importantly, hedonic behaviors that are commonly diminished in depressed patients, such as the desire to eat and to engage in sexual activity, are controlled by an interaction between the hypothalamus and dopamine system (e.g. see Will et al., 2003b; Hull and Dominguez, 2007). Thus it is noteworthy that the hypothalamus sends dynorphin and orexin neural projections to the three major regions of this system: the ventral tegmental area (VTA), nucleus accumbens, and medial prefrontal cortex (mPFC) (e.g. Fallon et al., 1985; Peyron et al., 1998; Fadel and Deutch, 2002; Baldo et al., 2003). Each of these regions expresses dynorphin and orexin receptors (Marcus et al., 2001; Knoll and Carlezon, 2010) and is implicated in depression susceptibility (see Covington et al., 2010a). Importantly, activation of dynorphin and orexin mechanisms within each also modulates hedonic behavior (Bals-Kubik et al., 1993; Davis et al., 2009; Aston-Jones et al., 2010).

Studies suggest that orexins' hedonic effect in the VTA might be dampened in depression. VTA orexin release stimulates dopamine function and reward seeking (Aston-Jones et al., 2010). But evidence of diminished cerebrospinal orexin levels, reduced diurnal orexin fluctuations, and deficient VTA dopamine neuron function have all been seen in depressed patients; who commonly show anhedonia (American Psychiatric Association, 2000; Klimek et al., 2002; Salomon et al., 2003; Brundin et al., 2007). Narcolepsy patients also show a high incidence of depression and a diminished VTA reward function (Daniels et al., 2001; Ponz et al., 2010). They show a massive loss in hypothalamic orexin and dynorphin cell expression and cerebrospinal orexin levels as well (Peyron et al., 2000; Crocker et al., 2005).

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Abbreviations: BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; FST, forced swim test; mPFC, medial prefrontal cortex; RIA, radioimmunoassay; VTA, ventral tegmental area.

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Diminished hypothalamic orexin measures, including cell number, have similarly been observed in preclinical models of depression that are known to characterize anhedonia and apathy-like symptomology (Allard et al., 2004; Feng et al., 2008; Lutter et al., 2008a). The evidenced decrease in orexin cell number suggests that orexin and dynorphin are likely both diminished in the hypothalamus in depression, and at least orexin diminished in its VTA, accumbens or mPFC terminal regions. There is suggestive evidence in depression models that orexin might be altered in the VTA (Feng et al., 2008). Also, anhedonic depression models show dopamine hypofunction (Miczek et al., 2011a) as would be expected with VTA orexin decreases (Narita et al., 2007; Moorman and Aston-Jones, 2010). But this could also be expected with accumbens dynorphin increases (Shippenberg, 2009) which have been evidenced in some preclinical models but not others (Bjomebekk et al., 2005; Bergstrom et al., 2008; Rubino et al., 2008; Carr et al., 2010).

These orexin and dynorphin alterations could affect reward desire (Aston-Jones et al., 2010; Knoll and Carlezon, 2010). Orexin release in the hypothalamus, as well as orexin and dynorphin release in the VTA, stimulates reward seeking in animals (Singh and Desiraju, 1988; Hamilton and Bozarth, 1988; Mitchell and Stewart, 1990; Muschamp et al., 2007; Aston-Jones et al., 2010; Espana et al., 2010a). Thus, the orexin cell loss described in depression models could diminish reward desire. But the reinforcing or euphoric value of rewards might also be diminished in these models due to their enhanced accumbens dynorphin levels. Orexin or dynorphin administration in the accumbens is aversive to animals (Bals-Kubik et al., 1993; Terashvili et al., 2004; Sharf et al., 2008), as is dynorphin in the mPFC and dynorphin and orexin stress mechanisms in the VTA (Bals-Kubik et al., 1993; Hata et al., 2011).

Thus, changes in the dynamics between orexin and dynorphin function in depressed patients could cause their anhedonia symptomology by disrupting these distinct reward emotional processes. As the above findings suggest, anhedonic symptoms could be caused by an increase in accumbens aversion due to locally enhanced levels of either neuropeptide. But they could also be caused by a decrease in VTA-stimulated reward seeking due to locally diminished levels of either peptide in hypothalamic orexin cell projections to the region. But whether depression models show an imbalance in dynorphin and orexin expression between the hypothalamus and dopamine system is unknown.

This study used radioimmunoassay (RIA) to evaluate this possibility in the social defeat animal model of depression; at a time that anhedonic sexual disinterest was expressed. Due to the diminished hypothalamic orexin cell numbers described in this model and the known co-expression of dynorphin in these cells (Chou et al., 2001; Lutter et al., 2008a), we hypothesized that defeated animals would show orexin and dynorphin decreases in the hypothalamus. And since the VTA typically receives a preferentially rich orexin innervation compared to the mPFC and accumbens reward regions (Fadel and Deutch, 2002), we predicted that orexin decreases would also be seen in the VTA. Anhedonia, including sexual disinterest, would not be unexpected after such loss (e.g. Harris et al., 2005; Muschamp et al., 2007; Wang et al., 2009; Moorman and Aston-Jones, 2010; Espana et al., 2010b; McGregor et al., 2011; Thompson and Borgland, 2011). But since dynorphin primarily inhibits dopamine function in the VTA and accumbens, and is co-expressed in cell bodies within the VTA in addition to afferents to the region (Nestler and Carlezon, 2006; Shippenberg, 2009; Knoll and Carlezon, 2010; Panksepp and Watt, 2011), we predicted that basal dynorphin peptide levels would be normal or perhaps enhanced in these regions in this anhedonic depression model.

EXPERIMENTAL PROCEDURES

Fig. 1 provides a concise depiction of the experimental design used in this study.

Animals and housing

Male Long–Evans rats (N = 39, 8 weeks of age, Harlan, Indianapolis, IN, USA) were socially housed in a light- (12-h cycle, lights on 7 AM) and temperature-controlled colony room with food and water available *ad libitum*. After 3 days of acclimation to the colony room, they received once daily habituation to handling (1 min for 7 days) and two habituation sessions to the proximity test cages employed in this study (10 min each). They were then singly housed and left undisturbed for 28 days before being used as intruder animals or controls in the social defeat paradigm described below. Isolate housing is known to enhance the development and long-term expression of a depression-like phenotype in this paradigm (Ruis et al., 1999; de Jong et al., 2005). All animal procedures were carried out in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and the VA Animal Care and Use Committee.

An established cohort of male Long–Evans rats were used as aggressive resident rats in this study (N = 15; 6–9 months of age). These animals showed a dominant propensity to fight, pin, and occasionally bite a well-known submissive animal that was briefly placed into its homecage. Since Long–Evans males are naturally aggressive once mature as indicated by the literature provided by Harlan (Harlan, Indianapolis), and social isolation enhances aggressiveness, each animal within this cohort had been purchased at 2 months of age and singly housed for 4 months prior to its initial use. They were approximately 3–6 months older and 175–200 g heavier than the above experimental animals that were exposed to social defeat by this cohort.

Social defeat animal model of depression

The resident–intruder social defeat paradigm was chosen to model depression since it induces several different types of anhedonia-like symptoms including diminished sexual pursuit (see Nocjar and Panksepp, 2009; Miczek et al., 2011b). Rats that were used as intruder rats (N = 22) were placed in the homecage of an aggressive resident rat during five 30-min social defeat sessions. Control animals (N = 17) were given identically timed sham defeat sessions during which they were exposed to the empty homecage of one of the male aggressive resident rats. Each session was 48–72 h apart and videotaped. Under this social defeat methodology, intruder animals are typically pinned supine by the resident animal (i.e. defeated) within 5–8 min, but remain physically exposed to the aggressive resident animal throughout the 30-min social defeat session (e.g. McLaughlin et al., 2006; Nocjar and Panksepp, 2009). We have found that

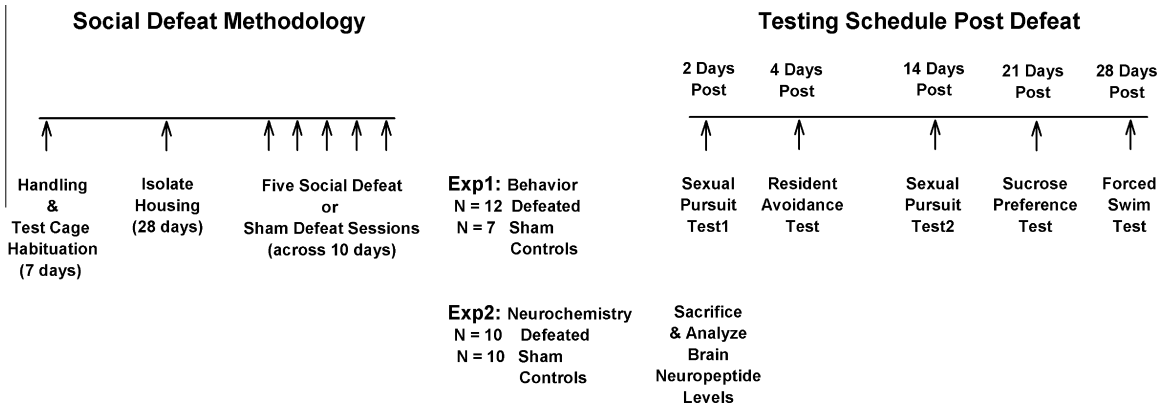

Fig. 1. Experimental design used in this study. Animals were first exposed to chronic social defeat as depicted in the left timeline. Starting 2 days after defeat terminated, they received several behavioral tests at the noted time points (Experiment 1), or they were sacrificed to determine brain neuropeptide expression (Experiment 2) as depicted in the right timeline.

the resident animal will not attack after the sessions initial fighting bouts if nearly continuous distanced submission is shown by the intruder in the form of frozen supine, upright or crouched postures. Thus after being quickly defeated and forced to submit, intruder animals are continuously stressed by the threat of physical aggression if they do not remain submissive across the 30-min session. Although intruder animals appeared psychologically stressed at the end of these sessions (e.g., they screeched and jumped from the resident's cage when the lid was removed), physical harm (scratches, bites, etc.) was rarely seen. Animals were removed from the study if they experienced significant harm such as scrotal bites, but not after minor harm (slight scratch, ear nip or toe-nail pull) that healed by the next defeat session and thus did not interfere with the animal's performance.

To show that submission developed in intruder animals, the following behaviors were assessed across defeat sessions: frozen crouch (hunched-back crouching distanced away from the resident rat or directly at the face of the resident rat), rough-and-tumbling fights, defensive uprights (animal upright, towards the resident rat, with 2-paws off of the cage surface), pins (intruder animal supine on its back below the resident rat or against the sidewalls), time to the first pin, and defensive guards (leg kicks or butt or arm push against the resident rat). These measures are typical social defeat assessments (Miczek, 1979; McLaughlin et al., 2006; Walker et al., 2009), except for our measure of frozen crouches directly at the face of the resident rat. In pilot work, we were surprised that some animals cautiously yet repeatedly approached the residents face and froze face-to-face. Because of the vulnerable proximity to the face of the resident animal, this behavior appears defiant while crouching away from the resident appears submissive. But the intruder many times remains frozen in its position if the resident walks away from the intruder at its face. Since others have shown that immobility oriented towards the resident was perhaps due to chronic stress-induced dynorphin release (McLaughlin et al., 2006), we hypothesized that these distinct crouch behaviors might differentially predict dynorphin and orexin neuropeptide change following defeat. Thus, both were measured and further assessed below.

Following social defeat termination, defeated and sham defeated animals were given several behavioral assessments (Experiment 1) or were sacrificed to assess brain neuropeptide levels (Experiment 2).

Experiment 1: Behavioral assessments

Our pilot evidence indicated that anhedonia was evidenced in socially defeated animals 2 days after defeat terminated (Nocjar and Panksepp, 2009). But we wanted to validate that our social defeat methodology was aversive and induced a lasting depression-like phenotype in animals. Thus, the following behavioral tests were administered to test this in seven of the above sham defeat control rats and in 12 that were socially-defeated as depicted in Fig. 1.

Sexual pursuit reward proximity test. To determine whether the social defeat methodology diminished reward desire, sexual interest was assessed 2 and 14 days after the last social defeat session. Sexual interest was assessed as in our previous reports (see Nocjar and Panksepp, 2002, 2007). In brief, rats were individually placed in a Plexiglas open-field reward proximity chamber that had a wire mesh stimulus cage located in each of its four corners. Two opposite corner stimulus cages contained a hormonally primed sexually-receptive female rat (10 μg estradiol benzoate, 48 h pretest, and 0.5 mg progesterone, 4 h pretest) or a non-receptive female. The stimulus cage wire mesh screening prevented copulation, but allowed assessment of a male animal's appetitive approach and investigative behavior towards each female target (e.g., time spent pawing and sniffing at the screening). The 10-min test was videotaped, and behavior was later tabulated by an individual that was blind to the animal's prior defeat experience. *Sexual preference scores* were calculated by subtracting the total time spent at the stimulus cage that contained the non-receptive female from the total time spent at the cage containing the sexually-receptive female. Since defeat can induce social avoidance (Krishnan et al., 2007; Lagace et al., 2010), time spent at both stimulus cages was used as an assessment of female social interest. Time at these cages plus the time spent at two additional empty stimulus cages in the remaining two corners of the proximity chamber was used as an assessment of stimulus cage exploratory interest, since a generalized stimuli disinterest could affect performance in this task. Vertical locomotion (number of vertical uprights [animal balanced upright on hind paws]) and horizontal locomotion (number of quadrant entries) were also counted across the session since locomotor alterations could also affect performance in this task. Note that chamber quadrants were clearly marked on the chambers floor.

Resident avoidance proximity test. Interest towards the aggressive male resident rat was assessed 4 days after the last social defeat or sham defeat session to see if defeated animals avoided the aggressive resident animal; indicative of conditioned fear. In brief, rats were exposed to a nearly identical reward proximity chamber and methodology as used in the sexual pursuit test above, except that this chamber had only two corner stimulus cages and the test stimuli differed. For this test, one stimulus cage was left empty while the other contained the specific aggressive resident rat that an animal had been physically ex-

posed to, or sham exposed to, during chronic social defeat sessions. The wire mesh screening allowed assessment of a male animal's appetitive approach and investigative behavior towards the aggressive resident animal (e.g., pawing and sniffing), while protecting it from any aggressive physical attack by the resident animal. *Resident preference scores* were calculated by subtracting the total time spent sniffing and pawing at the empty stimulus cage from the total time spent at the cage containing the aggressive resident animal. Time spent at both stimulus cages was used to assess stimulus cage exploratory interest. Locomotor behavior was also assessed as in the sexual pursuit task (see above).

Sucrose preference test. To test whether social defeat generally and persistently diminished reward desire, a 24-h homecage preference test for 1% sucrose versus plain water was also given to the above rats at approximately 21 days after their last social defeat or sham defeat session. Placement of the sucrose and plain water bottles was counterbalanced between animals. *Sucrose preference scores* were calculated by subtracting the total amount of water consumed from the amount of sucrose consumed. Prior to the test day, rats had been given a 2-h habituation session where they had free access to both liquids within their homecages.

Forced swim test (FST). FST immobility was also assessed in these same animals approximately 28 days after social defeat terminated. Immobility during this test is thought to model depression apathy-like symptomology. On the first day, rats were placed for 10 min in white buckets (51 cm diameter) that contained tepid water (30 °C, 40 cm deep (McLaughlin et al., 2006)) and their swimming behavior was videotaped. Twenty-four hours later, they were given a second swim test for 6 min. Videotapes were viewed blind, and time spent immobile (floating in the water and making only those movements necessary to keep the head above water), swimming (active swimming motions that moved the animal across the center or in circles within the center of the bucket), and climbing (forelimb thrashing movements directed against the sidewalls of the bucket) was tabulated, as well as immobility latency (time to first expression).

Experiment 2: Brain dynorphin-A, orexin-A and orexin-B neuropeptide assessment

Since altered orexin and dynorphin function is implicated in anhedonic behavioral expression (see introduction), and our preliminary findings indicated that anhedonic symptomology was evidenced in socially defeated animals when tested 2 days after the last social defeat session (Nocjar and Panksepp, 2009), we assessed orexin and dynorphin tissue levels at this time in this initial study. As described in Fig. 1, 10 socially defeated and 10 sham defeated animals that had received no other behavioral assessments were sacrificed 2 days post defeat. Their brains were removed and basal neuropeptide levels were determined in extracted tissue samples from the hypothalamus where orexin and dynorphin co-expressing cells are located, and from areas within the dopamine reward system where these cells project.

We used a tissue neuropeptide RIA technique to assess this (see below). RIA is commonly used in published reports of orexin alterations, whether detecting orexin in cerebrospinal fluid (CSF), brain microdialysate or brain tissue, though not in blood (Nishino, 2006). Tissue RIA does not provide a measure of neuropeptide release, but determines the level of available peptide within neuron cell bodies and/or within neuronal vesicles located in axons or axon terminals in a brain tissue sample. RIA is thought to be a sensitive measure, even of CSF orexin peptide levels, under the proper control procedures (Nishino, 2006). In fact, it is commonly thought that orexin A can be sensitively, specifically and reliably detected from all types of samples using either RIA or enzyme-linked immunosorbent assay (ELISA), but RIA is more sensitive than ELISA (Lin et al., 2002). And we have repeatedly detected orexin-B and orexin-A peptide levels, even detected their altered expression, in both cortical and subcortical brain tissue using RIA (Feng et al., 2007, 2008, 2009).

Tissue dissection. After decapitation, brains were quickly removed and sliced at 2-mm intervals with the aid of an ice-cold stainless steel brain matrix (ASI Instruments, Warren, MI, USA). Brain slices were placed on an ice-cold anodized aluminum block and the following brain regions were quickly extracted at the following anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) locations: mPFC (AP 3.7–1.7 mm; ML 0–1.0 mm; DV 0.0 to −5.0], nucleus accumbens [AP 1.7 to −0.3 mm; ML 0–2.5 mm; DV −5.5 to −9.0 mm], hypothalamus [AP −1.3 to −3.3 mm; ML 0–2.2 mm; DV −7.5 to −9.5 mm] and VTA [AP −5.3 to −7.3 mm; ML 0–1.0 mm; DV −7.5 to −8.5 mm]. Each tissue sample was immediately placed in pre-weighed plastic centrifuge tubes, weighed and then frozen at −80 °C until used for RIA neuropeptide assessment.

Since it is not known where orexin and dynorphin might interact within subregions of the mPFC, accumbens or VTA reward regions, the entire anterior to posterior and medial to lateral expanse of these three reward regions were extracted in this initial study before proceeding to potential subregions of interest within each. Thus, mPFC tissue samples included the entire anterior cingulate, prelimbic and infralimbic subregions. Within this sample were areas that are sensitive to stress, and in particular to stress-induced orexin-B attentional dysfunction (Lambe et al., 2005, 2007). Also included were regions of the anterior cingulate and ventral mPFC that become hypoactive after social defeat stress, and that when respectively lesioned or stimulated induces or reverses depression-like symptoms (Covington et al., 2005; Bissiere et al., 2006; Covington et al., 2010b; Kanarik et al., 2011). Although subregional specificity was lost with the inclusion of all three subregions in our sample, it allowed us to first determine whether neuropeptide change generally occurred in the mPFC after chronic defeat, before implementing studies of subregional involvement.

Nucleus accumbens samples included its shell and core subregions, as well as the medial olfactory tubercles. Care was taken to exclude surrounding areas that are involved in orexin-induced sleep/wake changes such as the medial septum (Berridge et al., 2010). VTA extraction encompassed its rostral and caudal regions, and included the small dopamine cell populations that are stimulated by orexin, and which project to the accumbens shell and mPFC (Vittoz et al., 2008).

Hypothalamus extraction encompassed the anterior hypothalamus back to the most anterior location of the posterior hypothalamus nucleus. Our sample included the lateral, dorsomedial and perifornical nuclei of the hypothalamus where orexin neurons are exclusively located (Nambu, 1999), and at levels where lateral and dorsomedial hypothalamic orexin cells are differentially activated by stress, food and addicting drug cues (see Aston-Jones et al., 2010). But it excluded surrounding areas that are involved in orexin-induced sleep/wake patterns such as the substantia innominata and medial preoptic area (Berridge et al., 2010), although the most rostral end of the sample might have included the most posterior border of the preoptic area.

Peptide extraction. Frozen tissue samples were processed using a well-established dry-sample protocol used in our lab for brain tissue peptide extraction (Feng et al., 2007, 2008). In brief, acetic acid (0.5 M) was added to each centrifuge tube containing sampled brain tissue, at a volume equal to 10 times the tissue weight. The centrifuge tubes were then moved to a boiling water bath for 10 min. After removing the tissue blocks, the microtubes were centrifuged for 30 s at 5500 rpm. The remaining supernatants (containing all peptides from the tissue sample) were

air-dried under a hood at 60 °C. The final dried peptide sample extracted from each tissue was subsequently stored at −80 °C until reconstituted for RIA neuropeptide assessment.

RIA assessment of dynorphin-A, orexin-A and orexin-B levels. Standard RIA kits for detecting orexin-A (#RK-003–30), orexin-B (#RK-003–32) and dynorphin-A (#RK-021–03) peptide expression were used (Phoenix Pharmaceuticals, Burlingame, CA, USA). Each of these kits provides highly reliable peptide specificity. The orexin-A kit for example does not cross react with orexin-B; and vice versa. Similarly, the dynorphin-A kit does not cross react with dynorphin-B, β endorphin, α-neo-endorphin or enkephalin and detects dynorphin-A in human, rat, mouse and porcine samples. We determined the quality of each kit before experimental assays were conducted by verifying (1) that the binding activity of 100 µl of the ^{125}I peptide solution was within a range of 8500–10 000 cpm and (2) that the kits sensitivity ratio was not less than 2.5. The sensitivity ratio was calculated by dividing the cpm value observed at the lowest detection concentration (1 pg/tube) by the cpm value observed at the highest detection concentration (128 pg/tube). Thus, $R = CPM_{1pg}/CPM_{128pg}$. For example, the mean sensitivity ratio for all orexin-A and -B RIA kits used in this study was 6.02 ± 0.50 and 2.72 ± 0.34, respectively; indicating that they provided a sensitive measure of tissue orexin levels.

Following these determinations, frozen dried peptide samples that had been extracted from the brain tissues collected in this study (see section 'Peptide extraction') were reconstituted in RIA buffer at a 1:50 dilution ratio (mg tissue dried peptide sample/µl RIA buffer). These sample stock solutions were then further diluted and preliminary assays were performed to determine the appropriate dilution ratio required to detect orexin-A, orexin-B and dynorphin-A within each of the brain regions assessed in this study. We found for example that the optimal dilution ratio for dynorphin-A was 1:50 in the mPFC, 1:75 in the nucleus accumbens, and 1:150 in the VTA and hypothalamus. Applying these ratios allowed us to sensitively detect dynorphin-A within tissue samples from all regions, although a more concentrated sample solution was required to detect it in mPFC samples for example than hypothalamic or VTA samples.

Once the optimal dilution ratios were determined, levels of all three neuropeptides were then independently determined within each brain tissue sample collected following the manufacturer's protocol for each neuropeptide (Phoenix Pharmaceuticals, Burlingame, CA, USA). After assay completion, the radioactivity of each sample tube (containing 100 µl of the optimal diluted sample) was determined with a gamma counter (Cobra II Auto-Gamma, Packard Instrument Company, Downers Grove, IL, USA). The sample value was compared to a standard curve assessed within the assay, which was generated using a standard protocol formulation. The indicated peptide level was then converted, based on the optimal dilution ratio used, to pg/mg tissue using GraphPad Prism software (San Diego, CA, USA). Since 100 µl of the optimal diluted sample was used in all peptide determinations, sample values were divided by two for example with a 1:50 optimal dilution ratio (mg tissue dried peptide sample/µl RIA buffer) since 2 mg of tissue was needed in 100 µl. Tissue sample assays were carried out in duplicate and the mean of these two measurements was used as data for statistical purposes.

Correlational assessment between hypothalamic dynorphin-A, orexin-A and orexin-B levels and prior social defeat behavioral expression. Immobility during chronic defeat requires dynorphin release (McLaughlin et al., 2006). And notably, defeat causes prolonged activation of orexin cells which typically co-express dynorphin (Chou et al., 2001; see Berridge et al., 2010). Their overstimulation also persistently and detrimentally alters their intracellular expression of orexin and dynorphin (Katsuki et al., 2010). Thus, we wanted to determine whether an animal's social defeat behavioral expression predicted hypothalamic orexin and dynorphin alteration. To assess this, frozen crouches, fights, uprights and guards that were expressed during the final social defeat session were each correlated with the above hypothalamic orexin-A, orexin-B and dynorphin-A measures.

Statistics

Data are presented as mean ± SEM. Significance was set at $p < 0.05$. To determine whether submission developed in animals across social defeat sessions, a dependent sample *t*-test was employed to assess whether submissive and aggressive behavioral expression changed between the first and last social defeat sessions. Then to determine the effects of social defeat on behavior (Experiment 1) and on brain neuropeptide expression (Experiment 2), independent sample *t*-tests and two-way repeated ANOVAs were used.

Independent sample *t*-tests were employed in Experiment 1 to determine whether defeated versus sham defeated animals differed in the resident avoidance test (resident preference scores, locomotor scores and stimulus cage exploration), sucrose preference test, or last FST (immobility, swimming and climbing duration). They were also used in Experiment 2 to determine whether these groups differed in neuropeptide expression.

Repeated measures ANOVAs were used when multiple tests of a behavior were being compared between these groups. Thus, a two-way repeated measures ANOVA (day × group) was used to determine whether defeated versus sham defeated animals differed during the first and second sexual pursuit tests (sexual pursuit scores, female social interest, motor exploration, and stimulus cage exploration), or differed across the two FSTs (% of session immobile, swimming and climbing) or four body weight tests (weight gain post defeat). If a significant interaction between test day and group was found, post-hoc analysis of main effects was further explored using post-hoc pairwise comparisons adjusted for multiple comparisons ($p <$ [0.05 divided by the number of comparisons]).

And finally, to determine whether an animal's social defeat behavioral expression predicted hypothalamic orexin and dynorphin peptide changes, Pearson's product correlations (r) were conducted between social defeat behaviors (crouching behaviors, fights, uprights and guards) and subsequent hypothalamic neuropeptide expression (orexin-A, orexin-B and dynorphin-A). The proportion of the variance in neuropeptide level attributable to each behavior (r^2) was also calculated.

RESULTS

Chronic social defeat behavior

As seen in the left graph in Fig. 2a, intruder animals spent more time in a frozen submissive crouch posture while in the presence of the aggressive resident animal by the final defeat session ($t[21] = 4.79$, $p < 0.001$; see Total Frozen Crouch). Two types of frozen crouch behavior were assessed in animals as depicted in the right half of this graph (for their behavioral description, see section 'Social defeat animal model of depression'). Frozen crouch postures distanced away from the resident animal increased across defeat sessions ($t[21] = 3.79$, $p < 0.01$), while crouch behaviors at the face of the resident animal increased minimally across defeat ($p = 0.20$). The right graph in Fig. 2a shows that animals also engaged in fewer fights ($t[21] = 3.48$, $p < 0.003$), defensive uprights ($t[21] = 2.52$, $p < 0.03$) and guards ($t[21] = 3.34$, $p < 0.004$) by the last defeat session. Thus, behavioral submission in the presence of the aggressive resident animal increased across social defeat sessions.

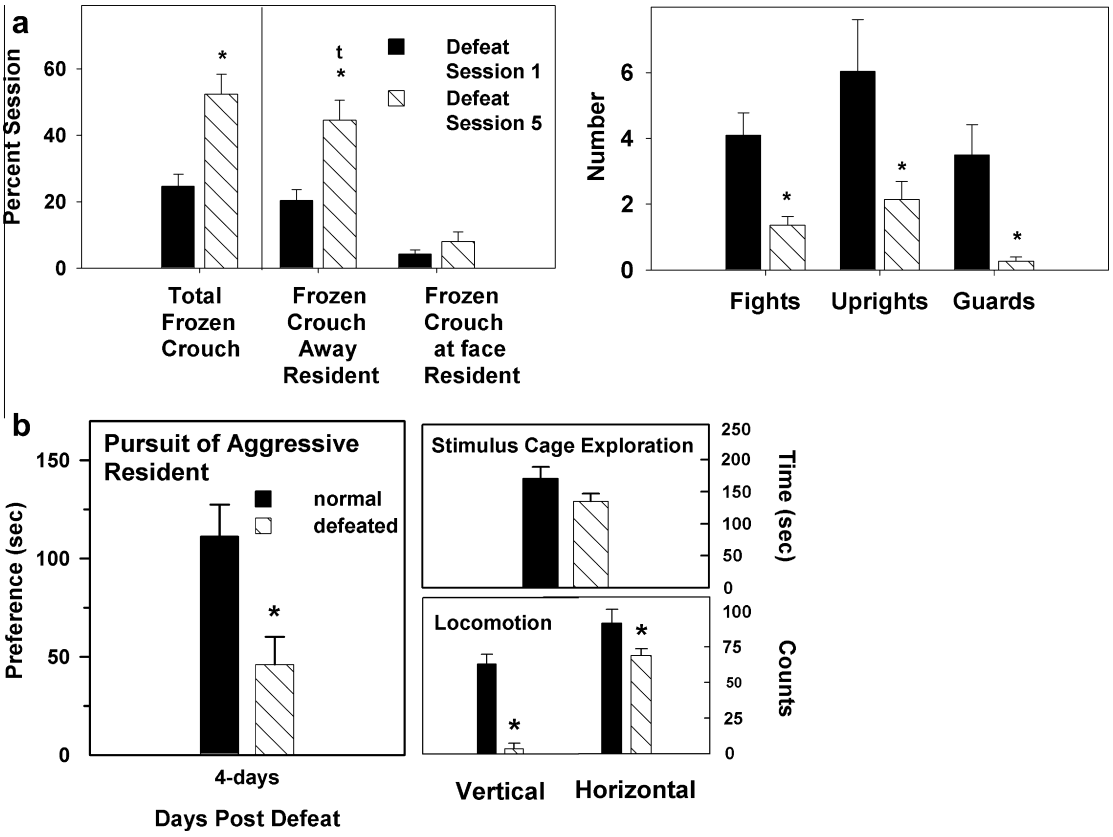

Fig. 2. Immediate and lasting behavioral response of socially defeated intruder rats to the aggressive resident animal. The resident/intruder social defeat paradigm was used to induce a depression-like phenotype in animals. Thus on five separate occasions, male rats (N = 22) were exposed to prolonged 30-min inescapable social defeat by an aggressive resident animal, each 2–3 days apart. (a) Submissive frozen crouch postures evidenced during the first and last social defeat sessions are illustrated (left graph), as well as the number of aggressive fights, defensive upright postures, and butt and paw guards (right graph). Note that sham control rats (N = 17) were given identically timed sham defeat sessions during which they were exposed to the resident rat's empty homecage. (b) Interest towards the aggressive male resident animal (4 days after defeat) by defeated rats (N = 12) and normal sham controls (N = 7) in experiment 1 (left graph). Time spent exploring all stimulus cages during the test as well as locomotor counts (# vertical uprights and horizontal quadrant entries) are shown in the right graph. All data are mean ± SEM. $^{*}p < 0.05$ compared to defeat session 1 (a) or to normal sham controls (b). ^{t}Crouch behavior that was distanced away from the resident rat negatively correlated to an animals' hypothalamic orexin and dynorphin levels measured 2 days later (see Fig 6b).

Note that frozen crouch distanced away from the resident animal was related to hypothalamic neuropeptide decreases seen 2 days after defeat terminated (see section 'Relationship between social defeat behavioral expression and hypothalamic levels' below and Fig. 6b).

Experiment 1: Behavioral study test performance

Sexual pursuit tests conducted 2 and 14 days after defeat. As seen in the left graph in Fig. 3a, sexual pursuit was diminished in defeated animals 2 days and 14 days after the last social defeat exposure compared to normal controls (overall group effect: $F[1,17] = 9.101$, $p < 0.009$; and no interaction with test day: $F[1,17] = 0.57$, $p = 0.46$). But as seen in the right graphs in Fig. 3a, defeated animals spent a similar amount of time investigating the two stimulus cages that contained the female animals as did normal controls (no overall group difference in social interest). And post-hoc analysis of a significant group × test-day interaction ($F[1,17] = 4.95$, $p < 0.05$) indicated that they did not differ from normal controls in social interest either 2-days ($t[17] = 0.94$, $p > 0.025$) or 14-days post defeat ($t[17] = 2.01$, $p > 0.025$). Also, their exploration of the stimulus cages during the test was similar to controls (no overall group effect $F[1,17] = 3.99$, $p > 0.05$; and no group × day interaction) as was their vertical and horizontal locomotion (no overall group effects: $F[1,17] = 2.14$, $p > 0.05$ and $F[1,17] = 0.04$, $p > 0.05$, respectively; and no group × day interaction in either test), as seen in the right graphs in Fig. 3a. These findings indicate that the diminished interest shown by defeated rats towards the sexually receptive female animal was not due to a decreased interest in the stimulus cage, a locomotor decrement or a decreased social interest towards females during this test. Thus, social defeat induced lasting sexual anhedonia in animals.

Resident avoidance proximity test conducted 4 days after defeat. Fig. 2b shows the interest expressed by defeated and normal sham defeated controls towards the aggressive resident animal when assessed 4 days after defeat (left graph). Opposite to controls, defeated animals avoided the stimulus cage that contained the aggressive

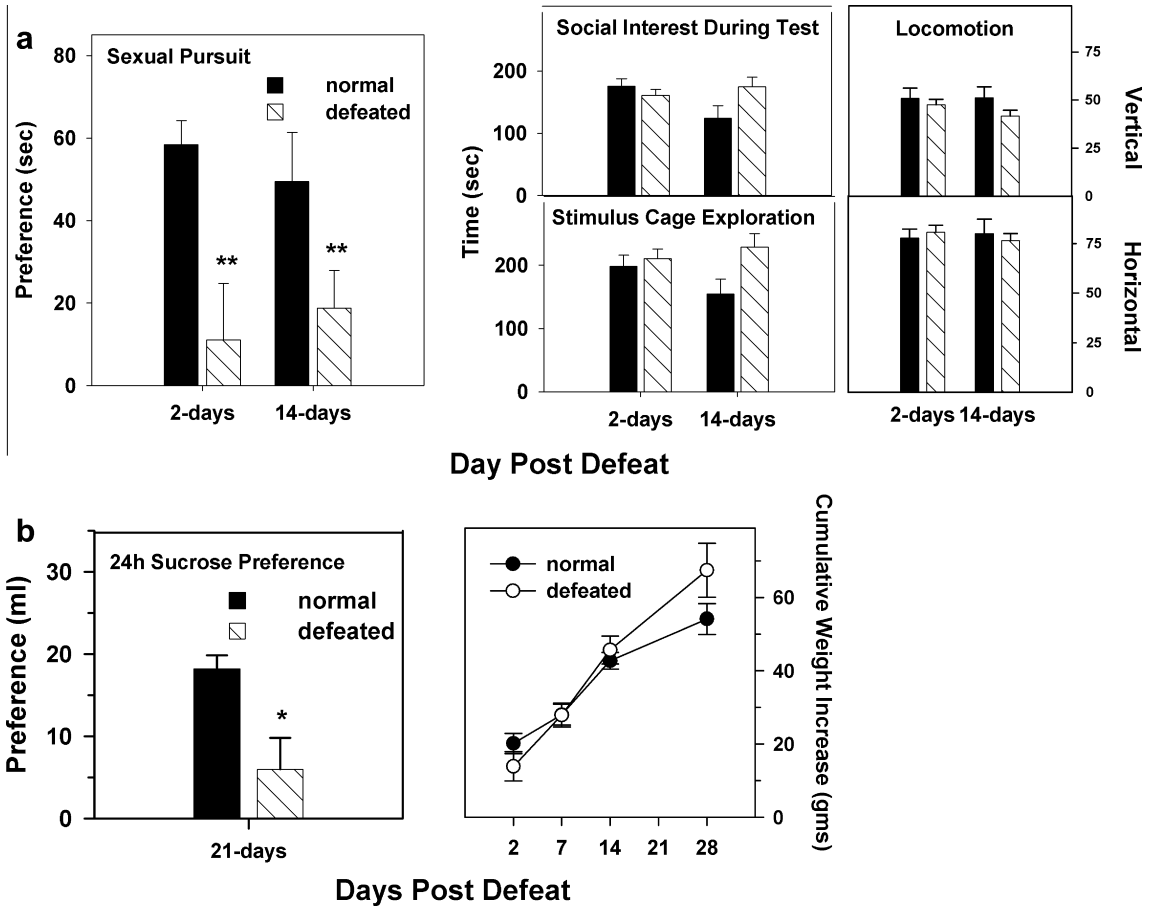

Fig. 3. Reward pursuit shown by socially defeated and normal sham defeated controls in Experiment 1. Three appetitive tests were given to animals to validate that the social defeat procedure induced lasting anhedonia-like behavior. (a) The left graph shows the sexual pursuit scores of the animals (see section 'Sexual pursuit reward proximity test') when tested 2 days and 14 days after the final social defeat session. These scores depict the male animal's preference to vigorously explore a screened stimulus cage that contained a sexually receptive female, over another that contained a non-receptive female, within a reward-proximity test box. The right graphs show that their sexual preference was not due to any change in social interest towards females, nor due to locomotor decreases. (b) The left graph depicts the 24-h preference shown by these same animals for a 1% sucrose solution over plain water, which was assessed in the animal's homecage 21 days after the final defeat session. The right graph shows their cumulative weight gain after chronic defeat sessions terminated (difference from pre-defeat weight). All data are mean ± SEM. $^{*}p < 0.05$, compared to normal sham controls. $^{**}p < 0.05$, overall ANOVA group effect which did not interact with test day.

resident rat that they had been exposed to during chronic defeat (t[17] = 2.93, $p < 0.01$). As indicated in the right graph in Fig. 2b, defeated animals did not spend significantly less time exploring the chambers two stimulus cages during the test (t[17] = 1.72, $p = 0.10$). But their vertical and horizontal locomotor counts were diminished during the test (t's [17] = 2.51 and 2.34 respectively, and p's < 0.03) in stark contrast to their normal locomotion shown during the sexual pursuit tests (see Fig. 3a). These findings of diminished approach towards the aggressive resident animal, of decreased open-field locomotor expression, but normal exploratory interest in the stimulus cages during the test, suggest that conditioned fear developed in defeated animals.

Sucrose preference test conducted 21 days after defeat. Fig. 3b shows that the 24-h sucrose preference scores of defeated animals were diminished compared to controls when tested 21 days after social defeat terminated (t[11] = 3.08, $p < 0.02$). Note that three of the initial animals in this study were sacrificed before this test was conducted, and the data from three additional animals were lost due to technical difficulties with the task. Nonetheless, this finding indicates that the anhedonia expressed by defeated animals when tested 2 days after social defeat (see sexual anhedonia expression in Fig. 3a) was still evident in this depression model nearly 3 weeks later.

As seen in the right graph in Fig. 3b, normal sham defeated controls and socially defeated animals gained weight after social defeat sessions terminated (overall day effect F[3,42] = 57.99, $p < 0.001$). But, defeated animals showed a non-significant trend towards faster weight gain than normal sham controls (group × weight interaction: F[3,42] = 2.65, $p = 0.06$).

FST conducted 28 days after defeat. Fig. 4 shows the immobility, swimming, and climbing behavior shown by defeated rats and normal sham defeated controls during two forced swimming tests that were conducted 24 h apart (FST1 and FST2) approximately 28 days after defeat

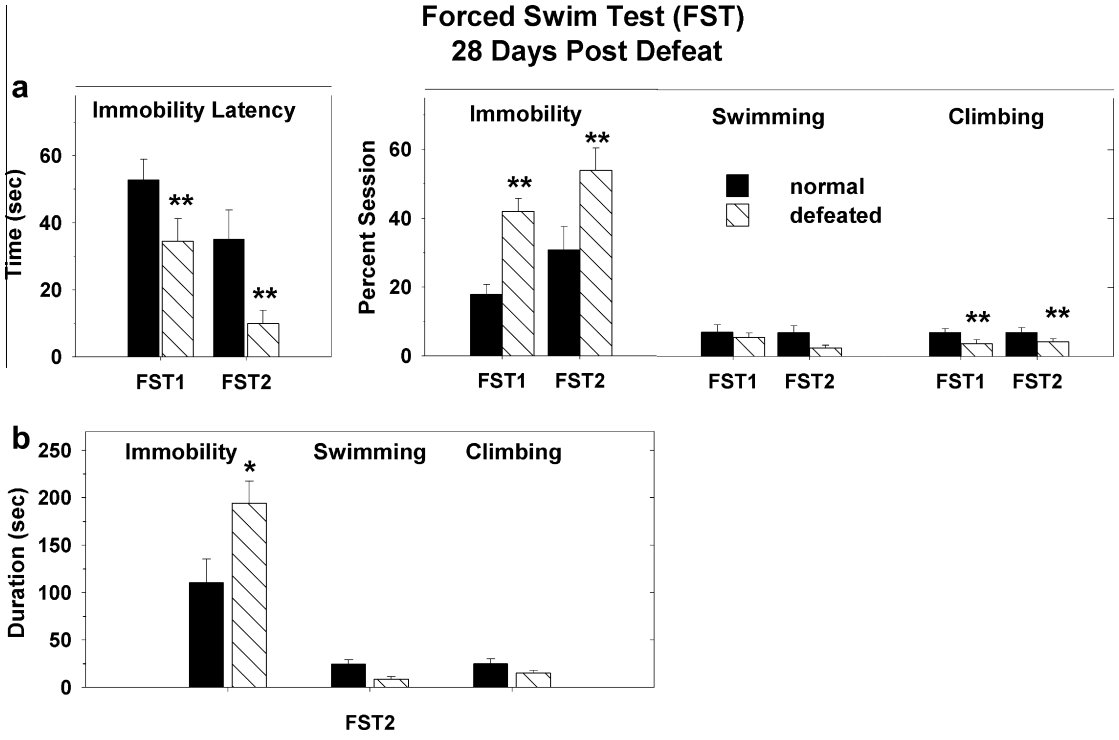

Fig. 4. FST behaviors shown by socially defeated and normal sham defeated controls in Experiment 1. Animals were tested 1 month after defeat terminated. Two FSTs were given 24 h apart (FST1 and FST2). (a) The left graph depicts how quickly animals became immobile. The right graph shows the percentage of time animals were immobile, swimming or climbing during these tests. (b) The graph shows immobility, swimming and climbing duration during the final FST (FST2) by defeated rats and normal controls. All data are mean ± SEM. $^{*}p < 0.05$, compared to normal sham-defeat controls. $^{**}p < 0.05$, overall ANOVA group effect which did not interact with test day.

terminated. As indicated in Fig. 4a (left graph), both defeated animals and normal controls showed a shorter latency to become immobile in FST2 than FST1 (overall test effect: $F[1,13] = 16.57$, $p < .01$; and no test × group interaction). But defeated rats showed shorter immobility latencies overall (overall group effect: $F[1,13] = 8.30$, $p < .02$; and no test × group interaction: $F[1,13] = 0.43$, $p = .52$).

As seen in the right graph in Fig. 4a, the percentage of time that defeated rats and normal controls spent immobile during these tests increased from FST1 to FST2 (overall test day effect: $F[1,13] = 8.49$, $p < .02$; and no test day × group interaction). But defeated animals showed a higher percentage of time immobile overall in both FST measures (overall group effect: $F[1,13] = 15.06$, $p < 0.003$; and no group × test interaction effect $F[1,13] = 0.01$, $p = 0.90$). And although the percentage of time spent swimming was not significantly different between defeated rats and normal controls (no overall group effect: $F[1,13] = 1.90$. $p = 0.19$; although the group × test interaction was marginal: $F[1,13] = 3.84$, $p = 0.07$ and an overall test effect was seen: $F[1,13] = 4.81$, $p < 0.05$), their percentage of time spent climbing during these tests did differ overall (overall group effect: $F[1,13] = 6.21$, $p < 0.03$; and no group - × test interaction or main test effect). Thus as typically seen in this paradigm, immobility was shown sooner and at a higher level in FST2. But defeated rats showed more immobility and a faster latency to become immobile. They also showed less climbing during these tests overall.

The actual duration of the above behaviors during FST2 is plotted in Fig. 4b. Immobility duration during FST2 differed between defeated and normal controls ($t[13] = 2.47$, $p < 0.03$). Also, non-significant trends of a diminished swimming duration ($t[13] = 2.08$, $p = 0.05$) and climbing duration ($t[13] = 1.74$, $p = 0.10$) was seen. Thus, socially defeated rats showed higher immobility in the final FST, and a trend towards lower swimming and climbing.

Note that the data were lost from one defeated animal due to a video-recording technical error during the test. And of course, data were not available from the same three defeated animals that were sacrificed prior to the sucrose test (see above).

Experiment 2: Neuropeptide study RIA determinations

The typical tissue extraction areas used in this study are depicted by the hatched regions in Fig. 5 (anterior extent of samples (Paxinos and Watson, 1998)). Samples were collected 2 days after the last sham or social defeat session since defeated animals typically show anhedonic sexual disinterest at this time (see Fig. 2; Nocjar and Panksepp, 2009).

RIA determinations of dynorphin-A, orexin-A and orexin-B within the mPFC, nucleus accumbens, VTA and hypothalamus. As seen in Fig. 6a, basal levels of orexin-A and orexin-B were diminished in the mPFC of socially de-

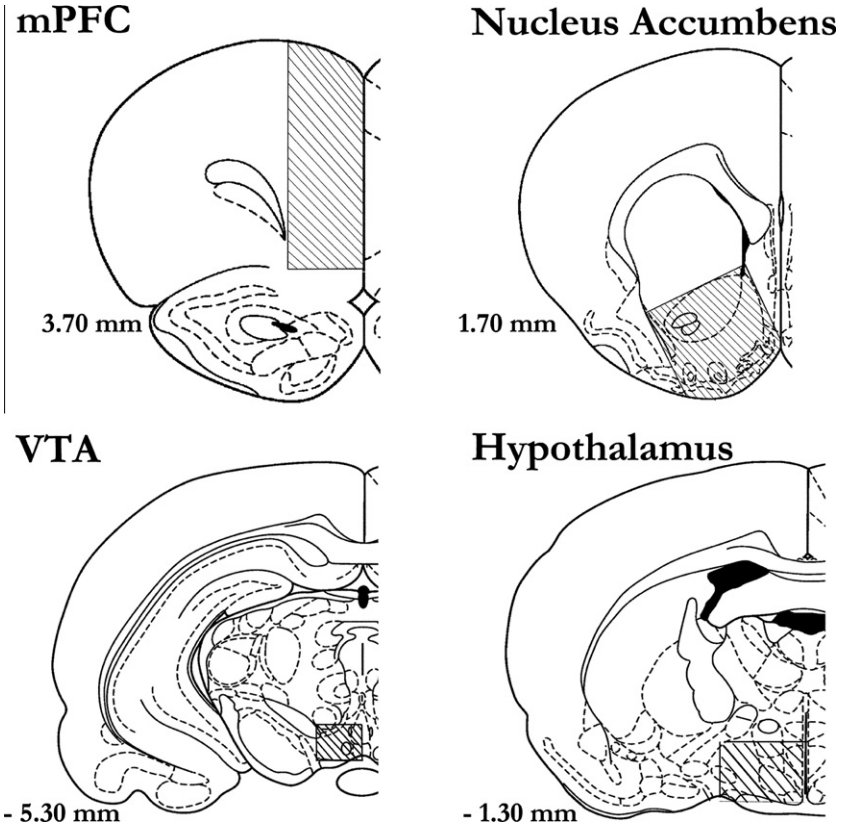

Fig. 5. The typical mPFC, nucleus accumbens, VTA and hypothalamus tissue extraction areas used in this study (see hatched areas). Each slice indicates the most rostral level of the sampled area, which was 2 mm in depth back through the brain. Orexin-A, orexin-B and dynorphin-A RIA neuropeptide assessments were conducted in each.

feated animals compared to sham defeated controls (*t*'s [13–14] = 2.24 and 3.29 respectively, *p*'s < 0.05); but dynorphin levels were not altered ($t[16] = 0.65$, $p = 0.95$). In the nucleus accumbens, persistent basal neuropeptide alterations were not evidenced (*t*'s [13–15] = 0.19, 0.15 and 0.33, *p*'s = 0.85, 0.88 and 0.71 respectively). In the VTA, basal levels of orexin-B were significantly diminished ($t[16] = 2.60$, $p < 0.02$) and a similar non-significant trend was apparent for orexin-A ($t[17] = 1.79$, $p = 0.09$), while dynorphin-A alterations were not evidenced ($t[18] = 0.63$, $p = 0.54$). However, dynorphin-A, orexin-A and orexin-B levels were all diminished in the hypothalamus of defeated animals (*t*'s [13–14] = 2.58, 2.19 and 3.53 respectively, *p*'s < 0.05). Thus, orexin-A and orexin-B were diminished in mesocortical regions of the dopamine reward system, and both orexin peptides as well as dynorphin-A were diminished in the hypothalamus in the social defeat model of depression.

Relationship between social defeat behavioral expression and hypothalamic levels of dynorphin-A, orexin-A and orexin-B neuropeptides. As seen in Fig. 6b, hypothalamic orexin-B levels shown two days after defeat terminated were negatively and significantly correlated with the animals distanced crouch behavior during defeat exposure (frozen crouch away from the resident animal, $r = -0.82$, $p = 0.04$). Although not shown for orexin-A ($r = -0.50$, $p = 0.24$, $r^2 = 0.25$), a similar non-significant trend was shown between dynorphin-A and this crouch behavior ($r = -0.68$, $p = 0.09$, $r^2 = 0.46$).

The relationship between hypothalamic neuropeptide expression and other social defeat behaviors was not significant: correlation coefficients between dynorphin-A, orexin-A or orexin-B and crouch at the residents face ($r = -0.10$, 0.40, and -0.11; respectively), fights ($r = -0.07$, 0.24, and -0.03; respectively), and uprights ($r = -0.26$, -0.17, and -0.30; respectively). And the proportion of the variance in neuropeptide level attributable to each of these behaviors (r^2) was 24% at best. Note that correlations with guard behaviors were not conducted because only one animal expressed this behavior during the final defeat session.

These findings indicate that a prolonged distanced crouch response predisposes orexin loss in this depression model. The similar trend between this behavior and dynorphin loss supports further exploration of this relationship since our hypothalamus tissue sample not only included orexin and dynorphin co-expressing cells in the dorsal hypothalamus, but also included other ventral hypothalamus dynorphin cell populations (Chou et al., 2001).

DISCUSSION

We found anhedonic sexual disinterest in the rat social defeat model of depression when assessed 2-days after termination of defeat. Importantly, orexin peptide levels were diminished in the dopamine reward system at this time and both orexin and dynorphin levels were decreased in the hypothalamus. The model also showed a lasting generalized depressive phenotype. Sexual pursuit

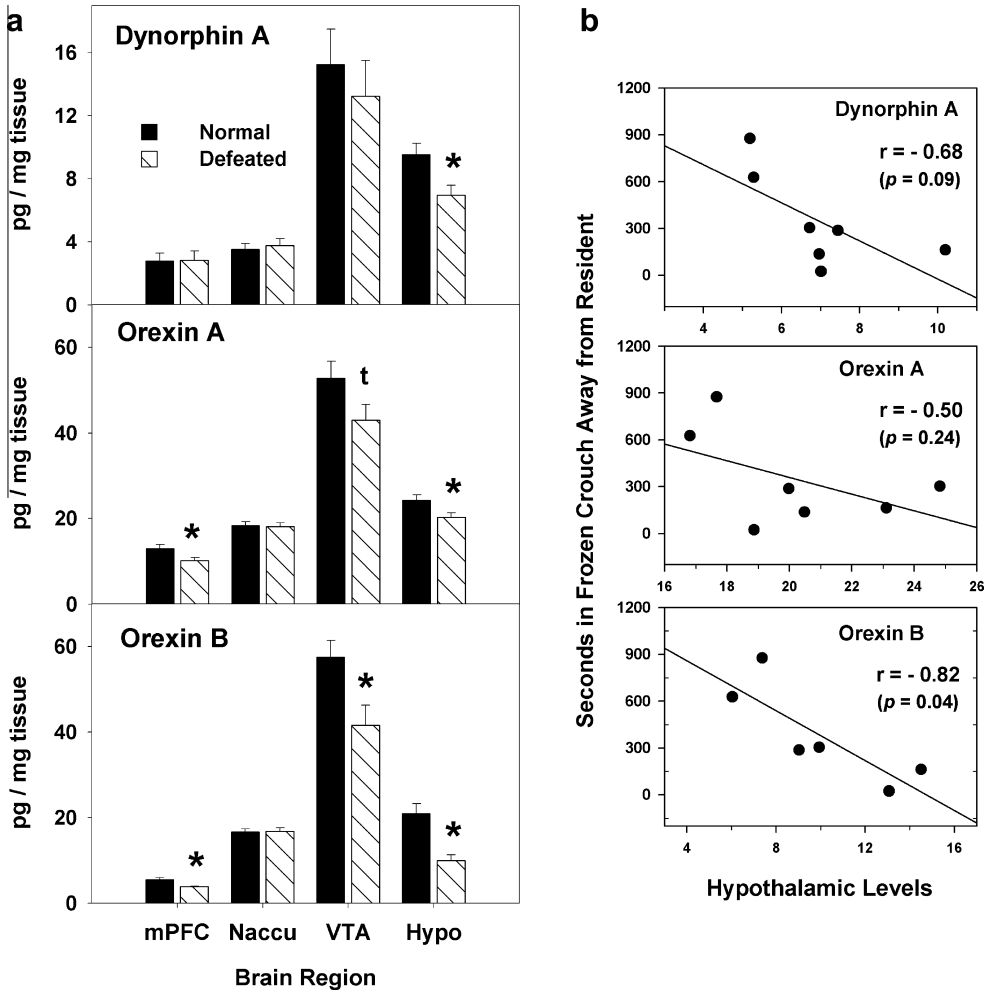

Fig. 6. Neuropeptide levels within the hypothalamus and dopamine reward system in socially defeated and normal sham defeated controls in Experiment 2. Two days after defeat terminated, socially defeated rats and normal sham controls ($N = 10$ each group) were sacrificed. Tissue samples from the mPFC, nucleus accumbens (Naccu), VTA and hypothalamus (Hypo) were collected using the extraction parameters described in Fig 5. Due to the exclusion of samples that extended an areas border, RIA analysis of the mPFC and Naccu included tissue from 7–9 animals from each group, VTA analysis included 9–10 from each and hypothalamus analysis included 6–9 from each. (a) RIA determinations of dynorphin-A, orexin-A and orexin-B peptide expression within these regions are shown. All data are mean (±SEM) pg peptide level per mg tissue sample collected from the area. $^{*}p < 0.05$ and $^{t}p = 0.09$, compared to normal sham controls. (b) Correlational data depicting the percentage of time spent in a distanced frozen crouch position during the final social defeat session and their basal hypothalamic dynorphin-A, orexin-A and orexin-B peptide levels expressed 2 days later. The Pearson Product Correlation Coefficient (r) and its significance (p) are also shown.

and sucrose intake remained diminished for at least 3 weeks, and apathy-like behavior in the FST was still evident one month after defeat terminated. This is the first report of diminished sexual interest or motivation in a depression model other than our preliminary findings (Nocjar and Panksepp, 2009), although enhanced FST apathy and diminished sucrose intake and copulation have all been previously seen (e.g. see Sugiura et al., 1997; Rygula et al., 2005; Lutter et al., 2008b; Haenisch et al., 2009; Ito et al., 2009; Miczek et al., 2011b). Our findings also provide the first direct evidence of a potential orexin dysfunction in mesocortical regions of the dopamine system in depression.

Orexin and dynorphin levels were diminished in the hypothalamus

Although stress can acutely enhance hypothalamic orexin cell activation (Winsky-Sommerer et al., 2004; Harris and Aston-Jones, 2006; Furlong et al., 2009; Berridge et al., 2010; Johnson et al., 2010; Nollet et al., 2011), a growing literature with depression animal models suggests that orexin cell function is likely diminished in depression. In the social defeat model used in the current work, decreased hypothalamic pre-proorexin mRNA and orexin cell count and activation have all been reported (Lutter et al., 2008a). And we show that orexin-A and orexin-B peptide levels are diminished. Hypothalamic decreases in orexin peptide and orexin cell size have also been seen in the neonatal clomipramine and Wistar–Kyoto depression models (Allard et al., 2004; Feng et al., 2008). Hypothalamic orexin$_1$ receptor expression is also diminished (Allard et al., 2004). And we provide the first evidence in preclinical depression models that both orexin and dynorphin peptides are decreased in the hypothalamus, which is noteworthy since dynorphin is co-expressed in nearly all hypothalamic orexin cells (Chou et al., 2001).

Further work is needed however to determine the location of the dynorphin loss since our hypothalamic tissue sample included orexin cells as well as other ventral hypothalamic cell populations that contain dynorphin (see Chou et al., 2001; Harthoom et al., 2005). But several pieces of evidence suggest that dynorphin was likely lost from orexin cells. First, our defeated animals showed higher weight increases than controls after defeat sessions terminated; an effect seen with orexin cell ablation and loss of both peptides (orexin/ataxin-3 transgenic mice (Nishino et al., 2000; Hara et al., 2001; Mieda et al., 2004; Crocker et al., 2005)) but not when orexin is lost but dynorphin remains in these cells (i.e., orexin deficient mice (Willie et al., 2001)). Second, orexin cell decreases have been previously reported in socially defeated animals (Lutter et al., 2008a). Third, prolonged activation of orexin neurons, as would occur under prolonged defeat stress (see Berridge et al., 2010), diminishes both orexin and dynorphin in hypothalamic orexin cells (Katsuki et al., 2010). And finally, we found that sexual pursuit and hypothalamic orexin and dynorphin peptide levels were all diminished 2 days after defeat terminated. In a similar temporally related fashion, castration decreases copulation and orexin cell survival which would ablate both orexin and dynorphin (Chou et al., 2001; Muschamp et al., 2007).

The hypothalamic orexin and dynorphin decreases seen in our social defeat depression model suggest that hypothalamic function may be vastly dysregulated in depression. Decreased hypothalamic orexin function diminishes reward seeking (Aston-Jones et al., 2010), and our depression model expressed anhedonia. But locally decreased levels of orexin and dynorphin could also dampen the activity of hypothalamic neuropeptide Y cells and cells that contain melanin concentrating hormone (Li and van den Pol, 2006); disturbing feeding (Dryden et al., 1996; Tritos et al., 2001; Chen et al., 2002; Chaffer and Morris, 2002; Bayer et al., 2002; Li and van den Pol, 2006). The diminished dynorphin levels could also disinhibit the remaining functional orexin cells (Li and van den Pol, 2006), dysregulating sleep and arousal, metabolism and energy balance (Horvath et al., 1999; Hagan et al., 1999; Bourgin et al., 2000; Seeley and Woods, 2003). Notably, disturbances in all have been seen in depression.

Interestingly, chronic social defeat induces immobility in animals by enhancing dynorphin release (McLaughlin et al., 2006). Although it is not known whether hypothalamic orexin and dynorphin co-expressing cells are involved in this dynorphin effect, chronic defeat causes prolonged activation of these cells which detrimentally affects their intracellular dynorphin and orexin expression (Berridge et al., 2010; Katsuki et al., 2010). Thus, we hypothesized that social defeat behavior might predict hypothalamic orexin and dynorphin loss in this study. Behaviors that were rarely shown during chronic defeat (fighting, uprights, guards and crouches at the residents face) did not predict neuropeptide loss likely due to low variability caused by their rare expression. But an animal's propensity to submissively crouch distanced from the resident animal predicted orexin loss in this depression model. A significant relationship with dynorphin loss was not seen (-0.68, $p = 0.09$), but this was perhaps due to the inclusion of other ventral hypothalamus dynorphin cell populations in our sample. Further assessment of this relationship should be conducted.

Orexin levels in mesocortical regions of the dopamine system were diminished

Social distress predisposes depression in humans and animals alike (Bjorkqvist, 2001; Huhman, 2006; see Miczek et al., 2011a) and appears to have a strong detrimental effect on dopaminergic brain regions. Several of the disturbances reported implicate orexin dysfunction.

For example, dampened mPFC function has been seen in anhedonic socially defeated animals and in depressed patients (e.g. see Covington et al., 2005; Mayberg et al., 2005; Bissiere et al., 2006; Konarski et al., 2007; Covington et al., 2010b; Kanarik et al., 2011). Cognitive and memory deficits (von Frijtag et al., 2000; Narayanan et al., 2011; Yu et al., 2011), as well as altered mPFC gliogenesis (Czeh et al., 2007), diminished brain-derived neurotrophic factor (BDNF) expression (Miczek et al., 2008), chromatin remodeling (Hinwood et al., 2011), and diminished pyramidal excitation and synaptic neuroplasticity have also been reported (Covington et al., 2005; Leussis and Andersen, 2008). We show that this model has diminished mPFC levels of orexin-A and orexin-B. Locally decreased orexin-B function could cause the cognitive, pyramidal and neuroplastic deficits and dampened mPFC function described in this model (see Huang et al., 2006; Borgland et al., 2006; Wise, 2006; Lambe et al., 2007). By triggering glutamate release, orexin-B enhances excitability of pyramidal cells in the mPFC (Lambe and Aghajanian, 2003; Lambe et al., 2005; Lambe et al., 2007). And notably, pyramidal cell stimulation in the ventral mPFC diminishes depressive symptoms in animals, while their inhibition in the rostral anterior cingulate induces these symptoms (Bissiere et al., 2006; Covington et al., 2010b).

And our demonstrated mPFC orexin-A decreases could at least partially cause anhedonia in this depression model. Orexin-A has been implicated locally in reward pursuit (Davis et al., 2009) and it stimulates deep neuronal layers of the mPFC that are implicated in reward seeking (McFarland and Kalivas, 2001; Bayer et al., 2004; Xia et al., 2005). Note that we hypothesized that anhedonia could also be caused by enhanced mPFC levels of dynorphin, since it locally induces dysphoria in animals (Bals-Kubik et al., 1993). But normal levels of the peptide were expressed in the area as seen also in the prefrontal cortex of depressed patients (Peckys and Hurd, 2001).

VTA hypofunction has also been seen in anhedonic socially defeated animals (Miczek et al., 2011a) similar to other stress-induced depression models and depressed patients (Di Chiara and Tanda, 1997; Klimek et al., 2002). Diminished BDNF expression is also seen in the area (Miczek et al., 2008). Note that prolonged uncontrollable or continuous social distress appears necessary to induce both anhedonia and dampened BDNF and VTA function in the social defeat depression model; milder exposures to weeks of brief rescued social distress

actually enhances reward seeking and VTA function (Miczek et al., 2008, 2011a). Our findings provide a potential mechanism for the VTA hypofunction evidenced in this model and for the anhedonia it would induce.

For example, the hypothalamus sends a prominent orexin projection to the VTA which when stimulated promotes effort and reward motivation, including sexual interest (Muschamp et al., 2007; Aston-Jones et al., 2010; example reviews Espana et al., 2010a; Thompson and Borgland, 2011). Orexin directly activates dopamine cells in this region, although perhaps mainly by an extrasynaptic mechanism (Narita et al., 2006; Balcita-Pedicino and Sesack, 2007; Vittoz et al., 2008). Orexin also magnifies glutamatergic drive to the area (Borgland et al., 2008; Moorman and Aston-Jones, 2010). Particularly important is orexin's diurnal amplification of mPFC glutamatergic stimulation of the VTA; an effect thought to diurnally promote motivational arousal (Moorman and Aston-Jones, 2010). Although dynorphin levels appeared minimally affected, our sexually-anhedonic defeated animals showed diminished VTA orexin expression which the above evidence indicates could cause VTA hypofunction and anhedonia symptomology.

In fact, several pieces of evidence implicate VTA orexin hypofunction in the sexual disinterest evidenced in depression. For example, orexin activates mesolimbic dopamine cells that are typically stimulated by exposure to an estrous female (Pfaus et al., 1990; Narita et al., 2006). Activation of these cells also purportedly triggers sexual pursuit (Mas et al., 1990; Louilot et al., 1991; Damsma et al., 1992; Hull and Dominguez, 2007); and orexin simultaneously activates these cells and stimulates sexual pursuit (Muschamp et al., 2007). Thus, VTA orexin loss could induce sexual anhedonia in depression by causing dopamine hypofunction. In support of this, we found decreased VTA orexin levels in a sexually-anhedonic depression model that expresses VTA dopamine hypofunction (Miczek et al., 2011a) and a copulatory disinterest that is reinstated by dopamine treatment (Sugiura et al., 1997).

A final hypothesis that was proposed in this study was that orexin and dynorphin enhancements might be seen in the accumbens in anhedonic socially defeated animals. Dynorphin locally inhibits accumbens dopamine release (see Nestler and Carlezon, 2006; Knoll and Carlezon, 2010; Alcaro and Panksepp, 2011). And notably dopamine hypofunction is seen in the area in this depression model (Miczek et al., 2011a). Furthermore, both orexin and dynorphin appear to induce dysphoria in the accumbens (Bals-Kubik et al., 1993; Terashvili et al., 2004; Sharf et al., 2008). Although evidence suggests that anhedonia could be due to changes in kappa receptor sensitivity or post-synaptic influences of dynorphin within the accumbens (Bruchas et al., 2007; Mu et al., 2011), we show that it is likely not due to changes in basal levels of dynorphin or orexin in the area.

Basal accumbens orexin changes have not been assessed in depression models prior to this study. But several labs have assessed dynorphin alterations. Unless animals were female, most concur with our finding of unaltered basal dynorphin in the area. This was shown regardless of whether the depressive-like phenotype was a natural genetic trait or induced by adolescent drug exposure or by chronic mild stress in adulthood (Bjomebekk et al., 2005; Bergstrom et al., 2008; Rubino et al., 2008). The dynorphin measure also did not matter. Prodynorphin mRNA was normal within soma throughout the accumbens in two of these depression models (Bjomebekk et al., 2005; Bergstrom et al., 2008). Also, RIA determination of accumbens tissue neuropeptide levels, which was used in the current study, showed normal dynorphin A in male anhedonic animals as we found (Rubino et al., 2008). Thus, dynorphin synthesis within accumbens cell soma as well as dynorphin-A peptide within local cells and afferents to the region appear normal in depression models.

But ELISA neuropeptide assessment detected accumbens dynorphin-A enhancements in the Wistar–Kyoto depression model (Carr et al., 2010), and similar to RIA, ELISA detects neuropeptide levels throughout the cell. Perhaps accumbens dynorphin enhancement is specific to the Wistar–Kyoto model, which differs from most depression models in its resistance to antidepressant treatment (Lopez-Rubalcava and Lucki, 2000; Tejani-Butt et al., 2003; Will et al., 2003a).

Conclusion and relevance to depression

This study demonstrates that an imbalance in orexin and dynorphin affective interactions between the hypothalamus and dopamine system may exist in depression. Our described mPFC and VTA orexin loss in the social defeat depression model indicates that orexin cell populations to mesocortical regions of the dopamine system may be particularly sensitive to social distress. Also, since such orexin loss could dampen both stimulus-induced and diurnal motivational arousal (Moorman and Aston-Jones, 2010), it implicates mesocortical orexin dysfunction in the anhedonia and apathy expressed by this preclinical model and in depressed patients. The orexin and dynorphin loss we describe in the hypothalamus could also cause extensive emotional dysregulation.

Unfortunately, little is known about orexin and dynorphin affective interactions. Further work that deciphers the behavioral effect of these imbalances may clarify their interactive role in brain emotional processing related to depression. Subregional assessment of the areas addressed in this study is also needed to better localize these roles. However, we do know that orexin functional decreases can cause emotional instability (Scott et al., 2011) as clearly seen in our orexin-deficient socially defeated animals. Orexin functional increases also ameliorate depressive-like symptomology in preclinical depression models and depressed patients (DeMet et al., 1999; Lutter et al., 2008a; Ito et al., 2009). Although it will be challenging to determine how our reported orexin and dynorphin decreases parallel or compound other effects seen in this depression model including numerous gene and neuropeptide synthesis alterations (Panksepp and Watt, 2011; Miczek et al., 2011b), more effective depression treatment will not be developed without further understanding of such multifactorial interactions.

CONTRIBUTORS

Drs. Nocjar and Panksepp contributed to the experimental design, while all authors contributed to the analysis, interpretation of the results and in the writing of the manuscript. Dr. Nocjar was responsible for conducting all animal behavioral work and brain tissue extraction, and Drs. Zhang and Feng for conducting the RIA analysis.

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REFERENCES

Alcaro A, Panksepp J (2011) The SEEKING mind: primal neuro-affective substrates for appetitive incentive states and their pathological dynamics in addictions and depression. Neurosci Biobehav Rev 35:1805–1820.

Allard JS, Tizabi Y, Shaffery JP, Trouth CO, Manaye K (2004) Stereological analysis of the hypothalamic hypocretin/orexin neurons in an animal model of depression. Neuropeptides 38:311–315.

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders. 1st ed. Washington, DC: American Psychiatric Press.

Aston-Jones G, Smith RJ, Sartor GC, Moorman DE, Massi L, Tahsili-Fahadan P, Richardson KA (2010) Lateral hypothalamic orexin hypocretin neurons: a role in reward-seeking and addiction. Brain Res 1314:74–90.

Balcita-Pedicino JJ, Sesack SR (2007) Orexin axons in the rat ventral tegmental area synapse infrequently onto dopamine and gamma-aminobutyric acid neurons. J Comp Neurol 503:668–684.

Baldo BA, Daniel RA, Berridge CW, Kelley AE (2003) Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. J Comp Neurol 464:220–237.

Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS (1993) Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J Pharmacol Exp Ther 264:489–495.

Bayer L, Mairet-Coello G, Risold PY, Griffond B (2002) Orexin/hypocretin neurons: chemical phenotype and possible interactions with melanin-concentrating hormone neurons. Regul Pept 104:33–39.

Bayer L, Serafin M, Eggermann E, Saint-Mleux B, Machard D, Jones BE, Muhlethaler M (2004) Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. J Neurosci 24:6761–6764.

Bergstrom A, Jayatissa MN, Mork A, Wiborg O (2008) Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. Brain Res 1196:41–52.

Berridge CW, Espana RA, Vittoz NM (2010) Hypocretin/orexin in arousal and stress. Brain Res 1314:91–102.

Bissiere S, McAllister KH, Olpe H-R, Cryan JF (2006) The rostral anterior cingulate cortex modulates depression but not anxiety-related behaviour in the rat. Beh Brain Res 175:195–199.

Bjomebekk A, Mathe AA, Brene S (2005) The antidepressant effect of running is associated with increased hippocampal cell proliferation. Int J Neuropsychopharmacol 8:357–368.

Bjorkqvist K (2001) Social defeat as a stressor in humans. Physiol Behav 73:435–442.

Borgland SL, Storm E, Bonci A (2008) Orexin B/hypocretin 2 increases glutamatergic transmission to ventral tegmental area neurons. Eur J Neurosci 28:1545–1556.

Borgland SL, Taha SA, Scart F, Fields HL, Bonci A (2006) Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. Neuron 49:589–601.

Bourgin P, Huitron-Resendiz S, Spier AD, Fabre V, Morte B, Criado JR, Sutcliffe JG, Henriksen SJ, de Lecea L (2000) Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. J Neurosci 20:7760–7765.

Bruchas MR, Land BB, Aita M, Xu M, Barot SK, Li S, Chavkin C (2007) Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. J Neurosci 27:11614–11623.

Brundin L, Bjorkqvist M, Petersen A, Traskman-Bendz L (2007) Reduced orexin levels in the cerebrospinal fluid of suicidal patients with major depressive disorder. Eur J Neuropsychopharm 17:574–579.

Carr GV, Bangasser DA, Bethea T, Young M, Valentino RJ, Lucki I (2010) Antidepressant-like effects of K-opioid receptor antagonists in Wistar Kyoto Rats. Neuropsychopharmacology 35:752–763.

Chaffer CL, Morris MJ (2002) The feeding response to melanin-concentrating hormone is attenuated by antagonism of the NPY Y1 receptor in the rat. Endocrinology 143:191–197.

Chen Y, Hu C, Hsu C-K, Zhang Q, Bi C, Asnicar M, Hsiung HM, Fox N, Slieker LJ, Yang DD, Heiman ML, Shi Y (2002) Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. Endocrinology 143:2469–2477.

Chou TC, Lee CE, Lu J, Elmquist JK, Hara J, Willie JT, Beuchmann CT, Chemelli RM, Sakurai T, Yanagisawa M, Saper CB, Scammell TE (2001) Orexin (hypocretin) neurons contain dynorphin. J Neurosci 21:RC168.

Covington III HE, Vialou V, Nestler EJ (2010a) From synapse to nucleus: novel targets for treating depression. Neuropharmacology 58:683–693.

Covington HEI, Kikusui T, Goodhue J, Nikulina EM, Hammer RP, Miczek KA (2005) Brief social defeat stress: long-lasting effects on cocaine taking during a binge and zif268 mRNA expression in the amygdala and prefrontal cortex. Neuropsychopharmacology 30:310–321.

Covington HEI, Lobo MK, Maze I, Vialou V, Hyman JM, Zaman S, LaPlant Q, Mouzon E, Ghose S, Tamminga CA, Neve RL, Deisseroth K, Nestler EJ (2010b) Antidepressant effect of optogenetic stimulation of the medial prefrontal cortex. J Neuroscience 30:16082–16090.

Crocker A, Espana RA, Papadopoulou M, Saper CB, Faraco J, Sakurai T, Honda M, Mignot E, Scammell TE (2005) Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. Neurology 65:1184–1188.

Czeh B, Muller-Keuker JL, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E (2007) Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology 32:1490–1503.

Damsma G, Wenkstern DG, Pfaus JG, Phillips AG, Fibiger HC (1992) Sexual behaviour increases dopamine transmission in the nucleus accumbens and striatum of male rats. Comparison with novelty and locomotion. Behav Neurosci 106:181–191.

Daniels E, King MA, Smith IE, Shneerson JM (2001) Health-related quality of life in narcolepsy. J Sleep Res 10:75–81.

Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC (2009) Dominant rats are natural risk takers and display increased motivation for food reward. Neuroscience 162:23–30.

de Jong JG, van der Vegt BJ, Buwalda B, Koolhaas JM (2005) Social environment determines the long-term effects of social defeat. Physiol Behav 84:87–95.

DeMet EM, Chicz-DeMet A, Fallon JH, Sokolski KN (1999) Sleep deprivation therapy in depressive illness and Parkinson's disease. Prog Neuropsychopharm Biol Psychiatry 23:753–784.

Di Chiara G, Tanda G (1997) Blunting of reactivity of dopamine transmission to palatable food: a biochemical marker of anhedonia in the CMS model? Psychopharmacology 134:351–353.

Dryden S, Wang Q, Frankish HM, Williams G (1996) Differential effects of the 5-HT1B/2C receptor agonist mCPP and the 5-HT1A agonist flesinoxan on hypothalamic neuropepetide Y in the rat: evidence that NPY may mediate serotonin's effects on food intake. Peptides 17:943–949.

Eriksson KS, Sergeeva OA, Selbach O, Haas HL (2004) Orexin (hypocretin)/dynorphin neurons control GABAergic inputs to tuberomammillary neurons. Eur J Neurosci 19:1278–1284.

Espana RA, Melchior JR, Roberts DC, Jones SR (2010a) Hypocretin 1/orexin A in the ventral tegmental area enhances dopamine responses to cocaine and promotes cocaine self-administration. Psychopharmacology 214:415–426.

Espana RA, Oleson EB, Locke JL, Brookshire BR, Roberts DC, Jones SR (2010b) The hypocretin-orexin system regulates cocaine self-administration via actions on the mesolimbic dopamine system. Eur J Neurosci 31:336–348.

Fadel J, Deutch AY (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. Neuroscience 111:379–387.

Fallon JH, Leslie FM, Cone RI (1985) Dynorphin-containing pathways in the substantia nigra and ventral tegmentum: a double labeling study using combined immunofluorescence and retrograde tracing. Neuropeptides 5:457–460.

Feng P, Hu Y, Li D, Vurbic D, Fan H, Wang S, Strohl KP (2009) The effect of clomipramine on wake/sleep and orexinergic expression in rats. J Psychopharmacol 23:559–566.

Feng P, Vurbic D, Wu Z, Hu Y, Strohl KP (2008) Changes in brain orexin levels in a rat model of depression induced by neonatal administration of clomipramine. J Psychopharmacol 22:784–791.

Feng P, Vurbic D, Wu Z, Strohl K (2007) Brain orexins and wake regulation in rats exposed to maternal deprivation. Brain Res 1154C:163–172.

Furlong TM, Vianna DML, Liu L, Carrive P (2009) Hypocretin/orexin contributes to the expression of some but not all forms of stress and arousal. Eur J Neurosci 30:1603–1614.

Haenisch B, Bilkei-Gorzo A, Caron MG, Bonisch H (2009) Knockout of the norepinephrine transporter and pharmacologically diverse antidepressants prevent behavioral and brain neurotrophin alterations in two chronic stress models of depression. J Neurochem 111:403–416.

Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N (1999) Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci U S A 96:10911–10916.

Hamilton ME, Bozarth MA (1988) Feeding elicited by dynorphin (1–13) microinjections into the ventral tegmental area in rats. Life Sci 43:941–946.

Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30:345–354.

Harris GC, Aston-Jones G (2006) Arousal and reward: a dichotomy in orexin function. Trends Neurosci 29:571–577.

Harris GC, Wimmer M, Aston-Jones G (2005) A role for lateral hypothalamic orexin neurons in reward seeking. Nature 437:556–559.

Harthoom LF, Sane A, Nethe M, Van Heerikhuize JJ (2005) Multi-transcriptional profiling of melanin-concentrating hormone and orexin-containing neurons. Cell Mol Neurobiol 25:1209–1223.

Hata T, Chen J, Ebihara K, Date Y, Ishida Y, Nakahara D (2011) Intra-ventral tegmental area or intracerebroventricular orexin-A increases the intra-cranial self-stimulation threshold via activation of the corticotropin-releasing factor system in rats. Eur J Neurosci 34:816–826.

Hinwood M, Tynan RJ, Day TA, Walker FR (2011) Repeated social defeat selectively increases delta FosB expression and histone H3 acetylation in the infralimbic medial prefrontal cortex. Cereb Cortex 21:262–271.

Horvath TL, Peyron C, Diano S, Ivanov A, Aston-Jones G, Kilduff TS, van den Pol AN (1999) Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. J Comp Neurol 415:145–159.

Huang H, Ghosh P, van den Pol AN (2006) Prefrontal cortex-projecting glutamatergic thalamic paraventricular nucleus-excited by hypocretin: A feedforward circuit that may enhance cognitive arousal. J Neurophysiol 95:1656–1668.

Huhman KL (2006) Social conflict models: can they inform us about human psychopathology? Horm Behav 50:640–646.

Hull EM, Dominguez JM (2007) Sexual behavior in male rodents. Horm Behav 52:45–55.

Ito N, Yabe T, Nagai T, Oikawa T, Yamada H, Hanawa T (2009) A possible mechanism underlying an antidepressive-like effect of Kososan, a kampo medicine, via the hypothalamic orexinergic system in the stress-induced depression-like model mice. Biol Pharm Bull 32:1716–1722.

Johnson PL, Truitt W, Fitz SD, Minick PE, Dietrich A, Sanghani S, Traskman-Bendz L, Goddard AW, Brundin L, Skekhar A (2010) A key role for orexin in panic anxiety. Nat Med 16:111–115.

Kanarik M, Alttoa A, Matrov D, Koiv K, Sharp T, Panksepp J, Harro J (2011) Brain responses to chronic social defeat stress: effects on regional oxidative metabolism as a function of a hedonic trait, and gene expression in susceptible and resilient rats. Eur Neuropsychopharmacol 21:92–107.

Katsuki H, Kurosu S, Michinaga S, Hisatsune A, Isohama Y, Izumi Y, Kume T, Akaike A (2010) Depolarizing stimuli cause persistent and selective loss of orexin in rat hypothalamic slice culture. Peptides 31:1131–1138.

Klimek V, Schenck JE, Han H, Stockmeier CA, Ordway GA (2002) Dopaminergic abnormalities in amygdaloid nuclei in major depression: a postmortem study. Biol Psychiatry 52:740–748.

Knoll AT, Carlezon Jr WA (2010) Dynorphin, stress, and depression. Brain Res 1314:56–73.

Konarski JZ, Kennedy SH, McIntyre RS, Rafi-Tari S, Soczynska JK, Mayberg HS (2007) Relationship between regional brain metabolism, illness severity and age in depressed subjects. Psychiatr Res 155:203–210.

Krishnan V, Han M-H, Graham DL, Berton O, Renthal W, Russo SJ, LaPlant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch A, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131:391–404.

Lagace DC, Donovan MH, DeCarolis NA, Fambauch LA, Halhotra S, Berton O, Nestler EJ, Krishnan V, Eisch AJ (2010) Adult hippocampal neurogenesis is functionally important for stress-induced social avoidance. Proc Natl Acad Sci U S A 197:4436–4441.

Lambe EK, Aghajanian GK (2003) Hypocretin (orexin) induces calcium transients in single spines postsynaptic to identified thalamocortical boutons in prefrontal slice. Neuron 40:139–150.

Lambe EK, Liu RJ, Aghajanian GK (2007) Schizophrenia, hypocretin (orexin), and the thalamocortical activating system. Schizophr Bull 33:1284–1290.

Lambe EK, Olausson P, Horst NK, Taylor JR, Aghajanian GK (2005) Hypocretin and nicotine excite the same thalamocortical synapses in prefrontal cortex: correlation with improved attention in rat. J Neurosci 25:5225–5229.

Leussis MP, Andersen SL (2008) Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. Synapse 62:22–30.

Li Y, van den Pol AN (2006) Differential target-dependent actions of coexpressed inhibitory dynorphin and excitatory hypocretin/orexin neuropeptides. J Neurosci 26:13037–13047.

Lin L, Wisor J, Shiba T, Taheri S, Yanai K, Wurts S, Lin X, Vitaterna M, Takahashi J, Levenberg TW, Koehl M, Uhl G, Nishino S, Mignot E (2002) Measurement of hypocretin/orexin content in the mouse brain using an enzyme immunoassay: the effect of circadian time, age and genetic background. Peptides 23:2203–2211.

Lopez-Rubalcava C, Lucki I (2000) Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. Neuropsychopharmacology 22:191–199.

Louilot A, Gonzalez-Mora JL, Guadalupe T, Mas M (1991) Sex-related olfactory stimuli induce a selective increase in dopamine release in the nucleus accumbens of male rats. A voltammetric study. Brain Res 553:313–317.

Lutter M, Krishnan V, Russo SJ, Jung S, McClung CA, Nestler EJ (2008a) Orexin signaling mediates the antidepressant-like effect of calorie restriction. J Neurosci 28:3071–3075.

Lutter M, Sakata I, Osborne-Lawrence S, Robinsky SA, Anderson JG, Jung S, Birnbaum S, Yanagisawa M, Elmquist JK, Nestler EJ, Zigman JM (2008b) The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. Nat Neurosci 11:752–753.

Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, Elmquist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 435:6–25.

Mas M, Gonzalez-Mora JL, Louilot A, Sole C, Guadalupe T (1990) Increased dopamine release in the nucleus accumbens of copulating male rats as evidenced by in vivo voltammetry. Neurosci Lett 110:303–308.

Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH (2005) Deep brain stimulation for treatment-resistant depression. Neuron 45:651–660.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655–8663.

McGregor R, Wu M-F, Barber G, Ramanathan L, Siegel JM (2011) Highly specific role of hypocretin (orexin) neurons: differential activation as a function of diurnal phase, operant reinforcement versus operant avoidance and light level. J Neurosci 31:15455–15467.

McLaughlin JP, Li S, Valdez J, Chavkin TA, Chavkin C (2006) Social defeat stress-induced behavioral responses are mediated by the endogenous kappa opioid system. Neuropsychopharmacology 31:1241–1248.

Miczek KA (1979) A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. Psychopharmacology 60:253–259.

Miczek KA, Nikulina EM, Shimamoto A, Covington HEI (2011a) Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal dopamine caused by episodic versus continuous social stress in rats. J Neurosci 31:9848–9857.

Miczek KA, Nikulina EM, Takahashi A, Covington HEI, Yap JJ, Boyson CO, Shimamoto A, de Almeida RM (2011b) Gene expression in aminergic and peptidergic cells during aggression and defeat: relevance to violence, depression and drug abuse. Behav Genet 41:787–802.

Miczek KA, Yap JJ, Covington III HE (2008) Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. Pharmacol Ther 120:102–128.

Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M (2004) Orexin peptides prevent cataplexy and improve wakefullness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci U S A 101:4649–4654.

Mitchell JB, Stewart J (1990) Facilitation of sexual behaviors in the male rat associated with intra-VTA injections of opiates. Pharmacol Biochem Behav 35:643–650.

Moorman D, Aston-Jones G (2010) Orexin/hypocretin modulates response of ventral tegmental dopamine neurons to prefrontal activation: diurnal influences. J Neurosci 30:15585–15599.

Mu P, Neumann PA, Panksepp J, Schluter OM, Dong Y (2011) Exposure to cocaine alters dynorphin-mediated regulation of excitatory synaptic transmission in nucleus accumbens neurons. Biol Psychiatry 69:228–235.

Muschamp JW, Dominguez JM, Sato SM, Shen R-Y, Hull EM (2007) A role for hypocretin (orexin) in male sexual behavior. J Neurosci 27:2837–2845.

Nambu T (1999) Distribution of orexin neurons in the adult rat brain. Brain Res 827:243–260.

Narayanan V, Heiming RS, Jansen F, Leting J, Sachser N, Pape HC, Seidenbecher T (2011) Social defeat: impact on fear extinction and amygdala-prefrontal cortical theta synchrony in 5-HTT deficient mice. PLoS one 6:e22600.

Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, Sakurai T, Yanagisawa M, Nakamachi T, Shioda S, Suzuki T (2006) Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. J Neurosci 26:398–405.

Narita M, Nagumo Y, Miyatake M, Ikegami D, Kurashi K, Suzuki T (2007) Implication of protein kinase C in the orexin-induced elevation of extracellular dopamine levels and its rewarding effect. Eur J Neurosci 25:1537–1545.

Nestler EJ, Carlezon WA (2006) The mesolimbic dopamine reward circuit in depression. Biol Psychiatry 59:1151–1159.

Nishino S (2006) Hypocretin measurements in the CSF, and blood and brain tissue: basic and clinical applications. In: Nishino S, Sakurai T, editors. The orexin/hypocretin system: physiology and patholphysiology. Totowa: Humana Press. p. 73–82.

Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. Lancet 355:39–40.

Nocjar C, Panksepp J (2002) Chronic intermittent amphetamine pretreatment enhances future appetitive behavior for drug- and natural-reward: interaction with environmental variables. Behav Brain Res 128:189–203.

Nocjar C, Panksepp J (2007) Prior morphine experience induces long-term increases in social interest and in appetitive behavior for natural reward. Behav Brain Res 181:191–199.

Nocjar C, Panksepp J (2009) Brain dynorphin and orexin interactions in social-defeat induced depressive symptomology. Soc Neurosci Abstr Chicago, Illinois.

Nollet M, Gaillard P, Minier F, Tanti A, Belzung C, Leman S (2011) Activation of orexin neurons in dorsomedial/perifornical hypothalamus and antidepressant reversal in a rodent model of depression. Neuropharmacology 61:336–346.

Panksepp J, Watt D (2011) Why does depression hurt? Ancestral primary-process separation-distress (PANIC/GRIEF) and diminished brain reward (SEEKING) processes in the genesis of depressive affect. Psychiatry 74:5–13.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. San Diego: Academic.

Peckys D, Hurd YL (2001) Prodynorphin and kappa opioid receptor mRNA expression in the cingulate and prefrontal cortices of subjects diagnosed with schizophrenia or affective disorders. Brain Res Bull 55:619–624.

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 6:991–997.

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliff JG, Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 18:9996–10015.

Pfaus JG, Damsma G, Nomikos GG, Wenkstern DG, Blaha CD, Phillips AG, Fibiger HC (1990) Sexual behavior enhances central dopamine transmission in the male rat. Brain Res 530:345–348.

Ponz A, Khatami R, Poryazova R, Werth E, Boesiger P, Bassetti CL, Schwartz S (2010) Abnormal activity in reward brain circuits in human narcolepsy with cataplexy. Ann Neurol 67:190–200.

Rubino T, Vigano D, Realini N, Guidali C, Braida D, Capurro V, Castiglioni C, Cherubino F, Romualdi P, Candeletti S, Sala M,

Parolaro D (2008) Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. Neuropsychopharmacology 33:2760–2771.

Ruis MAW, te Brake JHA, Buwalda B, de Boer SF, Meerlo P, Korte SM, Blokhuis HJ, Koolhaas JM (1999) Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. Psychoneuroendocrinology 24:285–300.

Rygula R, Abumaria N, Flugge G, Fuchs E, Ruther E, Havemann-Reinecke U (2005) Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 162:127–134.

Salomon RM, Ripley B, Kennedy JS, Johnson B, Schmidt D, Zeitzer JM, Hishino S, Mignot E (2003) Diurnal variation of cerebrospinal fluid hypocretin-1 (orexin-A) levels in control and depressed subjects. Biol Psychiatry 54:96–104.

Scott MM, Marcus JN, Pettersen A, Birnbaum SG, Mochizuki T, Scammell TE, Nestler EJ, Elmquist JK, Lutter M (2011) Hcrtr1 and 2 signaling differentially regulates depression-like behaviors. Behav Brain Res 222:289–294.

Seeley RJ, Woods SC (2003) Monitoring of stored and available fuel by the CNS: implications for obesity. Nat Rev Neurosci 4:909.

Sharf R, Sarhan M, DiLeone RJ (2008) Orexin mediates the expression of precipitated morphine withdrawal and concurrent activation of the nucleus accumbens shell. Biol Psychiatry 64:175–183.

Shippenberg TS (2009) The dynorphin/kappa opioid receptor system: a new target for the treatment of addiction and affective disorders? Neuropsychopharm Rev 34:27.

Singh J, Desiraju T (1988) Differential effects of opioid peptides administered intracerebrally in loci of self-stimulation reward of lateral hypothalamus and ventral tegmental area–substantia nigra. NIDA Res Monogr 87:180–191.

Sugiura K, Yoshimura H, Yokoyama M (1997) An animal model of copulatory disorder induced by social stress in male mice. effects of apomorphine and L-dopa. Psychopharmacology 133:249–255.

Tejani-Butt S, Kluczynski J, Pare WP (2003) Strain-dependent modification of behavior following antidepressant treatment. Prog Neuropsychopharm Biol Psychiatry 27:7–14.

Terashvili M, Wu HE, Leitermann RJ, Hung KC, Clithero AD, Schwasinger ET, Tseng LF (2004) Differential conditioned place preference responses to endomorphin-1 and endomorphin-2 microinjected into the posterior nucleus accumbens shell and ventral tegmental area in the rat. J Pharmacol Exp Ther 309:816–824.

Thompson JL, Borgland SL (2011) A role for hypocretin/orexin in motivation. Behav Brain Res 217:446–453.

Tritos NA, Mastaitis JW, Kokkotou E, Maratos-Flier E (2001) Characterization of melanin concentrating hormone and preproorexin expression in the murine hypothalamus. Brain Res 895:160–166.

Vittoz NM, Schmeichel B, Berridge CW (2008) Hypocretin/orexin preferentially activates caudomedial ventral tegmental area dopamine neurons. Eur J Neurosci 28:1629–1640.

von Frijtag JC, Reijmers LGJE, van der Harst JE, Leus IE, van den Bos R, Spruijt BM (2000) Defeat followed by individual housing results in long-term impaired reward and cognition-related behaviours in rats. Behav Brain Res 117:137–146.

Walker FR, Masters LM, Dielenberg RA, Day TA (2009) Coping with defeat: acute glucocorticoid and forebrain responses to social defeat vary with defeat episode behaviour. Neuroscience 162:244–253.

Wang B, You Z-B, Wise RA (2009) Reinstatement of cocaine seeking by hypocretin (orexin) in the ventral tegmental area: independence from the local corticotropin-releasing factor network. Biol Psychiatry 65:857–862.

Will CC, Aird F, Redei EE (2003a) Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. Mol Psychiatry 8:925–932.

Will MJ, Franzblau EB, Kelley AE (2003b) Nucleus accumbens mu-opioids regulate intake of a high-fat diet via activation of a distributed brain network. J Neurosci 23:2882–2888.

Willie JT, Chemelli RM, Sinton CM, Yanagisawa M (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 24:429–458.

Winsky-Sommerer R, Yamanaka A, Diano S, Borok E, Roberts AJ, Sakurai T, Kilduff TS, Horvath TL, de Lecea L (2004) Interaction between the corticotropin-releasing factor system and hypocretins (orexins): A novel circuit mediating stress response. J Neurosci 24:11439–11448.

Wise RA (2006) A new peptide input to learning and addiction. Neuron 49(483):484.

Xia J, Chen X, Song C, Ye J, Yu A, Hu Z (2005) Postsynaptic excitation of prefrontal cortical pyramidal neurons by hypocretin-1/orexin A through the inhibition of potassium currents. J Neurosci Res 82:729–736.

Yu T, Guo M, Garza J, Rendon S, Sun X-L, Zhang W, Lu S-Y (2011) Cognitive and neural correlates of depression-like behaviour in socially defeated mice. An animal model of depression with cognitive dysfunction. Int J Neuropsychopharmacol 14:303–317.

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