Addiction and Depression in the Brain Reward System

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The brain reward system consists of the fundamental neural pathways involved in eliciting motivation and rewarding experiences. This neural network has striking neurochemical and morphological similarities between different species. Impaired function of the brain reward system is implicated in both depressive behavior and drug addiction, and comorbidity between these two states in humans has been statistically documented.

Our main goal is the study of the mechanisms by which the brain reward system affects mood and motivation, and the development of new tools to examine and affect neuronal processes at the root of depressive behavior and drug addiction. We use animal models for addiction and depressive behavior to study neurochemical and electrophysiological alterations in their brain reward system, and the effect of repeated electrical stimulation of specific reward-related brain sites on behavioral, neurochemical and electrophysiological outcomes.

Addiction represents the pathological usurpation of neural processes that normally serve reward-related learning, resulting in a strong habitual memory. Our neurochemical assays revealed that repeated exposure to cocaine induces long-term alterations in levels of glutamatergic receptors within subregions of the ventral tegmental area (VTA) and the nucleus accumbens (NAC), which are fundamental sites in the brain reward system. These long-lasting alterations can be viewed as an expression of brain plasticity induced by chronic drug use. Therefore, we study and try to address the issue of treating drug addiction as a problem of weakening or erasing the strong addictive memory trace. Intra-cranial electrical stimulation (ICS) and transcranial magnetic stimulation (TMS) have been shown to alter neuronal activity, synaptic transmission, plasticity and behavior. Previous studies using a pharmacological approach demonstrated that upon retrieval, established memories, like addiction, become labile and enter a transient state, in which they can be changed, weakened or erased. However, this pharmacological approach is of limited value in humans because of toxicity. To this end, we used acute and repeated ICS of reward related brain areas in rats and TMS in humans, immediately after retrieval of the addictive memory trace, to try and induce localized neuronal adaptations that may change, weaken or erase the addictive memory trace. Repeated electrical stimulation of either a major reward pathway in the lateral hypothalamus or in the prefrontal cortex (PFC) resulted in partial normalization of glutamatergic alterations reported above, and induced a reduction in drug seeking behavior as measured by the frequency of lever presses for the drug (Fig. 1). Furthermore, acute ICS of the basolateral amygdala, applied immediately after presentation of drug-associated cues, significantly reduced cocaine seeking behavior. Lastly, in humans, 10 daily sessions of repetitive TMS of the dorsolateral PFC, immediately after presentation of smoking-related cues, reduced cigarette consumption and cue-induced craving. Hence, stimulating reward-related brain regions might be a novel strategy for normalizing neuronal adaptations induced by repeated drug use and thereby treating addiction.

In our animal studies on addiction, we mainly use a standard model in which rats learn to press a lever to self-administer heroin or cocaine directly into an implanted intravenous catheter. We have recently further developed this model to assess relapse to drug use. Relapse in humans can be induced by exposure to drug-associated cues. The ability of drug cues to provoke 'relapse' and the associated neurochemical and electrophysiological outcomes have been studied in laboratory animals using a reinstatement model in which resumption of drug seeking is assessed after extinction of drug-reinforced responding. In this model, there are no adverse consequences to drug-seeking behavior. However, in humans, abstinence is often self-imposed, and relapse episodes likely involve making a choice between the desire for the drug and the negative consequences of pursuing it (a conflict situation). Thus, we created a conflict model of cue-induced relapse in rats that approximates the human condition. Rats were trained to

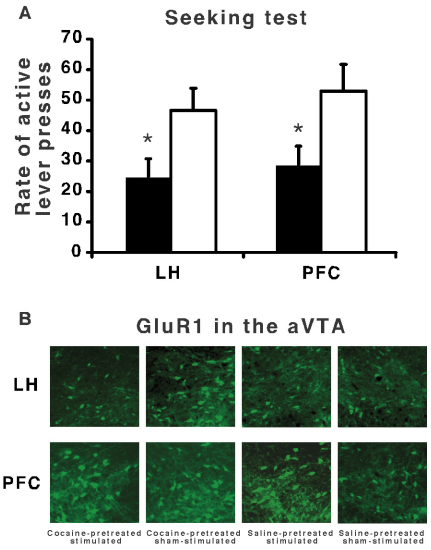

Fig 1. *Effects of ICS treatment of the lateral hypothalamus (LH) or the PFC on cocaine self-administration and GluR1 levels in the VTA. Each lever press provided 0.5 mg/kg cocaine i.v. Rats learned quickly to lever-press for cocaine and reached a maintenance criterion. Then ICS (black bars) or sham (white bars) treatment was given for 30 min/day in their home cages for 10 d. A) Average rate of active lever presses per hour performed during the reward-free seeking test conducted a day after termination of the ICS treatment. Data are presented as mean (± s.e.m) values. *P < 0.05 using two-way ANOVA followed by Fisher's PLSD post-hoc tests. B) Representative immunohistochemical images showing the effect of repeated cocaine and ICS of the LH or the PFC on GluR1 in the anterior VTA. (from Levy et al., 2007)*

lever press for cocaine and infusions were paired with a discrete light cue. An electric barrier was then introduced by electrifying the floor area near the levers. Responding decreased over days with increasing electrical intensities, until the rats did not approach the levers for 3 days. Subsequently, the effect of intermittent, non-contingent, light-cue presentations on resumption of lever response (relapse) was assessed, with the electric barrier remaining activated. Noncontingent cue exposure led to resumption of lever presses during the relapse tests in most rats (Fig. 2). Surprisingly, 24 h later, most rats resumed lever responding in a subsequent test without presentation of non-contingent drug cues. Large individual differences in responding were observed during both tests. At its current stage of development, the conflict relapse model appears particularly suitable for studying individual differences in cue-induced relapse to cocaine-seeking or factors that promote this relapse.

* different from the no noncontingent cue control condition, $p<0.05$; #different from the inactive lever, $p<0.05$ (From Cooper et al., 2007).

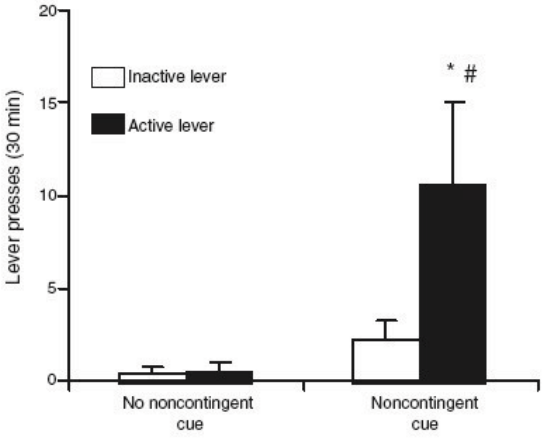

Fig 2. *Relapse to cocaine seeking induced by exposure to noncontingent cocaine cues, using the conflict model. After3 days of self-imposed abstinence induced by exposure to the electric barrier the effect of exposure to the drug cue (light above the active lever) was measured. Data are presented as the Mean±SEM number of lever presses on the previously active lever and on the inactive lever in two groups of rats that were either exposed or not to noncontingent cue presentations during a single test session.*

Our models for depression involve a battery of standard behavioral tests for motivation, hedonia and exploration, which are impaired in depressive states and expressed by various abnormalities in brain reward pathways. According to the neurotrophic hypothesis of depression, decreased levels of brain-derived neurotrophic factor (BDNF) and neurogenesis in the hippocampus play a critical role in depression. To test the role of neuroplasticity within reward pathways in depression, we are measuring these factors and other markers for brain plasticity in our different studies of depressive behavior. Despite the established association between reduced BDNF levels and neurogenesis in the hippocampus and depression, it is not known whether reduced BDNF levels can directly precipitate depressive behavior, or are merely a side effect of depression. Moreover, the specific brain sites in which BDNF is critical to depressive behavior are unknown, and direct evidence of neurogenesis impairment due to local reduction in BDNF levels does not exist *in-vivo.* Recently, we demonstrated that a reduction in BDNF expression in specific hippocampal subregions is not only associated with, but actually *causes* depressive behavior and reduced neurogenesis. We used RNA interference and lentiviral vectors to induce BDNF knockdown in specific brain sites such as the dentate gyrus (Fig 3), subiculum and CA3 of mature rat brains. We found the dentate gyrus (Fig 4), but not the CA3, to be an essential subregion for the development of depressive behavior. Furthermore, BDNF in the subiculum appears to be essential for normal hedonic-like behavior. We also found that BDNF reduction in the dentate gyrus directly impairs neurogenesis by reducing neuronal differentiation, but not proliferation. Our results provide significant new support for the neurotrophic hypothesis of depression by directly showing how local reduction of BDNF actually causes depressive behavior and impairs neurogenesis.

Depression is thought to result from a combination of genetic predisposition and environmental factors. However, despite the extensive biomedical research conducted in the field and identification of neurochemical abnormalities, including those in the reward system, we still lack basic information about the etiology and pathophysiology of depression. In order to study the genetic factors of depressive behavior under controlled conditions we are establishing a novel animal model for depression based on selective breeding for depressive phenotypes. Three different rat lines have been established in our lab by selective breading for depressive (DRL), normal (NRL) or motivated (MRL) behaviors, as measured by a battery of computerized behavioral tests. We are now testing the 10th generation and have found most aspects of motivation and hedonia to be hereditary in our model. DRL rats show consistent behavioral deficits characteristic of depression as compared to MRL and NRL rats. In addition, neurochemical analysis revealed that DRL rats express altered BDNF levels in reward-related brain regions relative to MRL and NRL rats. Moreover, we have recently found that electroconvulsive therapy, but not a standard antidepressant drug (desipramine) normalizes depressive behavior as well as BDNF levels in DRL rats. We believe that this new model for drug resistant depression will be useful in future studies of the genetic basis of depressive behavior.

A key factor in the environmental component of depression is chronic stress. Exposure to chronic mild stress (CMS) is known to induce anhedonia in adult animals, and is associated with the development of depression in humans. We have recently characterized the behavioral effects of CMS in young and adult animals and measured markers for alterations in neuronal

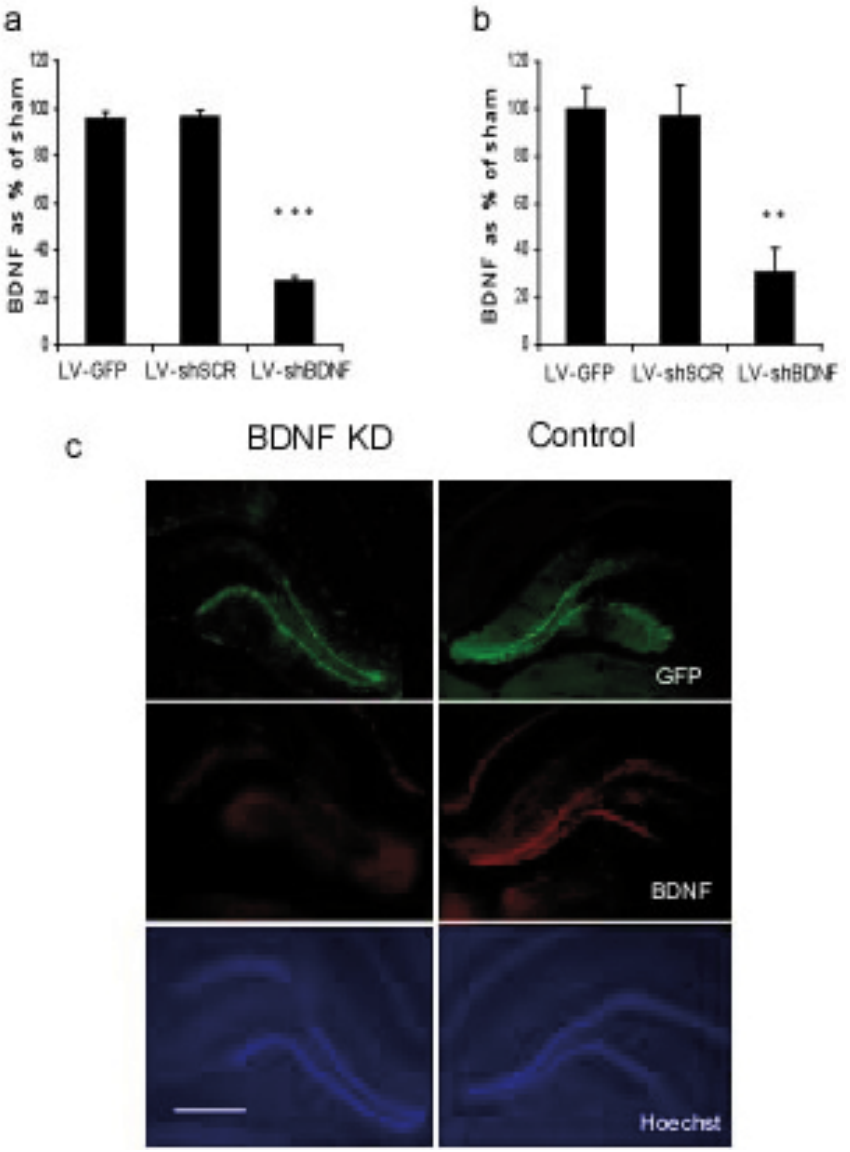

Fig 3. *In-vitro and in-vivo validation of LV-shBDNF. The effect of LV expressing GFP only (LV-GFP), scrambled shRNA (LV-shSCR) or shRNA complementary to rat's BDNF coding axon (LV-shBDNF) on BDNF expression was tested. (a) C6 cells were infected with the various LV and BDNF concentration was measured by ELISA. (b) The same vectors were injected into the dDG of the hippocampus of adult rats. Brain slices were observed under an epifluorescence microscope after staining for the BDNF protein using immunohistochemistry. BDNF levels are represented as a % of BDNF expression in brain slices from non infected rats. (c) Representative micrographs of the dDG of an adult rat injected with LV-shSCR (Control) or LV-shBDNF (BDNF KD). GFP was observed under a fluorescence microscope (upper). BDNF protein expression was observed by immunohistochemistry (middle). Cell nuclei were observed using Hoechst (lower), scale bar = 500μm. Values are mean + SEM (**P<0.01, ***P<0.001).*

plasticity induced by chronic stress. A growing body of data, including our recent findings, suggests that BDNF, neurogenesis and the glutamatergic system, which are known to play a major role in neuronal plasticity, are critical in the pathophysiology and treatment of mood disorders. We found that CMS induced anhedonia in adult but not in young animals, as measured by a set of behavioral paradigms. Furthermore, while CMS decreased BDNF levels and neurogenesis in the hippocampus of adult rats, it increased BDNF levels and neurogenesis in young rats. We also found that CMS altered AMPA receptor GluR1 subunit levels in the hippocampus and the NAC of adult, but not young rats. Therefore, chronic stress exerts substantially different neurochemical effects in young and adult animals that may explain our novel findings on the behavioral resilience of young animals to chronic stress.

To enhance neuroplasticity in the CMS model, we used brain stimulation to test for behavioral and neurochemical outcomes. Extensive brain stimulation such as electroconvulsive therapy (ECT) is known to be effective in treating depressive behavior and increases BDNF levels and neurogenesis in the hippocampus. Thus, we compared ECT with site-specific subconvulsive stimulation via behavioral testing, followed by BDNF-level measurement. Our aim was to test whether sub-convulsive stimulation using intracranial electrical stimulatin (ICS) of reward-related brain sites, such as the prelimbic cortex (PLC) or NAC, can induce antidepressant effects in a widely-used rat model for depressive behavior based on CMS. We utilized a battery of behavioral tests to assess the effectiveness of the various treatments, and measured levels of BDNF in brain sites reported to express altered BDNF levels in depression or in response to effective treatments. Repeated ICS of either the NAC or the ventral, but not the dorsal, PLC reversed the main behavioral deficit and the reduction of hippocampal BDNF levels, induced by CMS. This study implicates the ventral PLC and the NAC in the pathophysiology of depressive behavior and suggests that ICS of these regions can induce an antidepressant effect similar to ECT, without the cognitive impairment caused by the convulsive treatment.

In order to further examine plasticity-related alterations in the function of the brain reward system that are critically involved in depressive behavior and drug addiction, we used *in-vivo* electrophysiological recording. Specifically, we examined modifications in the ventral subiculum-nucleus

accumbens (vSub-NAc) pathway, which is implicated in processing of contextual information and motivational function. We recorded evoked potentials in the NAc in response to stimulation of vSub of the hippocampus in anesthetized animals. In response to paired-pulse stimulation and high frequency stimulation protocols, the vSub-NAc pathway demonstrated short-term plasticity. However, this pathway in naïve rats was not amenable to long-term potentiation (LTP). Surprisingly, in animals exposed to CMS and expressing depressive behaviors, the vSub-Nac pathway was sensitized as measured in a strength-duration curve, and LTP was induced in this pathway only in animals exposed to CMS but not in the normal controls and not in animals exposed to acute stress, or chronically exposed to an enriched environment. As this pathway processes environmental inputs to the reward system, our findings may indicate how exposure to chronic stress induces long-lasting susceptibility to environmental inputs. Interestingly, cocaine also induced metaplasticity in this pathway, as high frequency stimulation induced LTP in this pathway in cocaine, but not saline, treated animals.

In parallel, and based on some results obtained in the abovementioned projects, we are testing the efficacy of repeated stimulation of reward-related regions of the human brain in treating depressive disorders and addiction. These studies are done in collaboration with clinical centers. We have previously developed a novel coil for non-invasive transcranial magnetic stimulation of reward-related regions in the human brain and proved the ability of our approach to stimulate deep brain regions non-invasively and safely. Consistently with our animal studies using brain stimulation in the CMS model, we found in two different human studies, that deep transcranial stimulation of the prefrontal cortex exerts a potent antidepressant effects on patients who did not respond previously to antidepressant drugs. These studies will further enrich our knowledge of brain reward pathways and will be of paramount importance in designing novel treatments for depression and addiction.

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Acknowledgements

NIH (NIDA - R21 Grant), ISF, The National Institute for Psychobiology; The BIAL foundation.

A.Z. is an incumbent of the Joseph and Celia Reskin Career Development Chair.

INTERNAL support

The Rosenzweig-Coopersmith Foundation; Gerhard & Hannah Bacharach Fund; Yeda.