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Using In Vitro Electrophysiology to Screen Medications: Accumbal Plasticity as an Engram of Alcohol Dependence

R. Renteria, Z.M. Jeanes, R.A. Mangieri, E.Y. Maier, D.M. Kircher, T.R. Buske, and R.A. Morrisett¹

University of Texas at Austin, Austin, TX, United States

Abstract

The nucleus accumbens (NAc) is a central component of the mesocorticolimbic reward system. Increasing evidence strongly implicates long-term synaptic neuroadaptations in glutamatergic excitatory activity of the NAc shell and/or core medium spiny neurons in response to chronic drug and alcohol exposure. Such neuroadaptations likely play a critical role in the development and expression of drug-seeking behaviors. We have observed unique cell-type-specific bidirectional changes in NAc synaptic plasticity (metaplasticity) following acute and chronic intermittent ethanol exposure. Other investigators have also previously observed similar metaplasticity in the NAc following exposure to psychostimulants, opiates, and amazingly, even following an anhedonia-inducing experience. Considering that the proteome of the postsynaptic density likely contains hundreds of biochemicals, proteins and other components and regulators, we believe that there is a large number of potential molecular sites through which accumbal metaplasticity may be involved in chronic alcohol abuse. Many of our companion laboratories are now engaged in identifying and screening medications targeting candidate genes and its products previously linked to maladaptive alcohol phenotypes. We hypothesize that if manipulation of such target genes and their products change NAc plasticity, then that observation constitutes an important validation step for the development of novel therapeutics to treat alcohol dependence.

1. INTRODUCTION:THE NUCLEUS ACCUMBENS IN DRUG REWARD PROCESSING

The mesocorticolimbic system is composed of dopaminergic neurons of the ventral tegmental area (VTA) and its projection sites including the nucleus accumbens (NAc), prefrontal cortex, hippocampus, and amygdala (Sesack & Grace, 2010). The mesocorticolimbic system is often referred to as the reward system of the brain and is critical for reward and reinforcement processing, motivation, and goal-directed behaviors (Wise, 2004). It is well accepted that most drugs of abuse, including ethanol, activate the mesocorticolimbic dopamine system and lead to an increase in extracellular dopamine concentrations in the NAc (Di Chiara & Imperato, 1988; Doyon et al., 2003; Imperato & Di Chiara, 1986; Weiss, Lorang, Bloom, & Koob, 1993).

¹Corresponding author: ramorris@mail.utexas.edu.

Depending on the pharmacological class they belong to, all drugs of abuse elicit, to different extents, an incentive arousal state due to their ability to increase extracellular dopamine in the NAc shell. This incentive arousal state facilitates the rate of current instrumental behavior, the acquisition and expression of secondary reinforcement, and the reinstatement of previously extinguished instrumental responding. In addition, it facilitates the consolidation of mnemonic traces of salient stimuli that are associated with affective states (Di Chiara et al., 2004). In short, dopamine in the NAc alerts the animal to the significance of a certain stimulus, so that appropriate associations can be made between the surroundings and behaviors that preceded the result of that stimulus. Repeated noncontingent exposure to drugs of abuse is thought to cause maladaptive activation of dopamine transmission. This could result in aberrant motivational behaviors typical of addiction—compulsive perseverance on obtaining drugs and drug-related stimuli at the expense of more conventional nondrug rewards. Thus, although it had been believed that dopamine directly mediated the rewarding effects of natural and drug reinforcers, more recent hypotheses focus on the role of mesolimbic dopamine as (1) a motivational learning signal (Spanagel & Weiss, 1999); (2) a signal of pathological associative learning in addiction (Di Chiara, 2002); (3) a neural substrate of incentive salience (Robinson & Berridge, 2003); or (4) a signal that informs the predictability of reward-related cues associated with previous drug availability (Fiorillo, Tobler, & Schultz, 2003).

Medium spiny neurons of the NAc therefore function to integrate this aforementioned dopamine signal in the context of cognitive, sensory, and emotional states. As such, cortical neurons are the likely promoters of goal-directed behaviors, with (1) the ventral subiculum of the hippocampus providing spatial and contextual information; (2) the prefrontal cortex supplying executive control, including task switching and response inhibition; and (3) the basolateral and central amygdala communicating information regarding conditioned associations as well as affective drive (Ambroggi, Ishikawa, Fields, & Nicola, 2008; Gruber, Hussain, & O'Donnell, 2009; Ito, Robbins, Pennartz, & Everitt, 2008; Kalivas, Volkow, & Seamans, 2005; Wolf, 2002).

The NAc shell subregion, in particular, has been associated with aspects of drug reward (Carlezon, Devine, & Wise, 1995; Ikemoto, 2007; Rodd-Henricks, McKinzie, Li, Murphy, & McBride, 2002; Sellings & Clarke, 2003). Evidence suggests that the transition to an addicted state follows adaptations first in the NAc shell, then NAc core, and finally the dorsal striatum. In nonhuman primates, the NAc shell and core subregions are organized in a series of parallel circuits linked in an ascending spiral to the dorsal striatum in a manner that could account for the transition from goal-directed to habitual behaviors during the development of addiction (Haber, Fudge, & McFarland, 2000; Sesack & Grace, 2010). Neuroadaptations of the mesocorticolimbic system are thought to underlie both the development and expression of addiction to ethanol and a variety of other reinforcers (Luscher & Malenka, 2011). Thus, understanding the exact sequence and cell-type specificity of these synaptic plasticity changes, within the mesocorticolimbic system, is critical for formulating model systems to investigate new therapeutic targets.

2. FORMS OF SYNAPTIC PLASTICITY IN THE SHELL AND CORE OF THE NUCLEUS ACCUMBENS

The bidirectional ability of neurons to increase and decrease synaptic strength is referred to as synaptic metaplasticity. As such, plasticity of neural circuits allows for the structural and functional reorganization of synapses in response to different stimuli. Long-term depression (LTD) and long-term potentiation (LTP) are the best-characterized mechanisms for modulating synaptic strength in an experience-dependent manner—a long-lasting decrease or increase in synaptic strength, respectively. Both processes are thought to be involved in information storage, important in learning and memory, and other physiological processes. In the NAc, several distinct forms of plasticity have been described that can result in either a decrease or an increase in synaptic strength.

2.1 NMDA Receptor-Dependent Plasticity

NMDA receptor (NMDAR)-dependent LTP and LTD are two forms of synaptic plasticity that require coincident activity of pre- and postsynaptic neurons. At resting membrane potentials, NMDARs are blocked by Mg^{2+} . However with sufficient depolarization, the Mg^{2+} block is relieved and glutamate activation of NMDARs allows Ca^{2+} influx and subsequently activates intracellular signaling cascades that are responsible for altering synaptic strength.

The best-characterized form of postsynaptic plasticity in the NAc is NMDAR-dependent LTD (Thomas, Malenka, & Bonci, 2000). This form of LTD is induced by prolonged low-frequency stimulation (LFS, 1–3 Hz) of presynaptic terminals paired with postsynaptic membrane depolarization, resulting in weak activation of postsynaptic NMDARs. This leads to a moderate amount of calcium influx through NMDARs and this intermediate elevation in postsynaptic calcium results in the activation of calcineurin and protein phosphatase 1 (Mulkey, Herron, & Malenka, 1993). The decrease in synaptic strength is due to the removal of AMPA receptors (AMPA) from the postsynaptic membrane. Clathrin adaptor proteins bind to the C-terminus of the GluA2 subunit which leads to clathrin-mediated endocytosis of GluA2-containing AMPARs (Beattie et al., 2000; Malenka, 2003). The interaction of adaptor proteins with the GluA2 subunit is critical for the expression of NMDAR-dependent LTD (Brebner et al., 2005; Jeanes, Buske, & Morrisett, 2014; Scholz et al., 2010).

NMDAR-dependent LTP is induced by high-frequency stimulation (100 Hz) and results in stronger activation of NMDARs and a larger Ca^{2+} influx compared to the induction of LTD. The larger increase of post-synaptic Ca^{2+} leads to a distinct intracellular signaling cascade that includes the activation of Ca^{2+} -calmodulin kinase type II (CAMKII) ultimately resulting in the insertion of postsynaptic AMPARs (Malenka & Nicoll, 1999). NMDAR-dependent LTP has been described in the NAc of both rats and mice (Kombian & Malenka, 1994; Li & Kauer, 2004; Pascoli, Turiault, & Lüscher, 2012; Pennartz, Ameerun, Groenewegen, & Lopes da Silva, 1993; Schotanus & Chergui, 2008).

2.2 Endocannabinoid-Mediated Plasticity

Endocannabinoid (eCB)-mediated plasticity is well documented in the dorsal striatum (Lovinger & Mathur, 2012) and has been described in the NAc as well (Robbe, Kopf, Remaury, Bockaert, & Manzoni, 2002). The most common expression mechanism of eCB-mediated plasticity in the NAc involves activation of presynaptic CB1 receptors. CB1 receptors are G-protein-coupled receptors (GPCRs) and are one of the most abundant GPCRs expressed in the central nervous system. Activation of group 1 metabotropic glutamate receptors (mGluR_{1/5}), or a rise in postsynaptic Ca²⁺, leads to the production of eCBs, which then act as a retrograde signal and activate presynaptic CB1 receptors. Activation of CB1 receptors results in a G_{ai/o}-dependent reduction in adenylyl cyclase and protein kinase A activity that suppresses neurotransmitter release. Induction protocols for eCB LTD vary widely between brain regions (Heifets & Castillo, 2009). For example, a prolonged moderate stimulation (13 Hz) results in eCB-mediated LTD in the NAc (Hoffman, Oz, Caulder, & Lupica, 2003; Robbe et al., 2002).

Transient receptor potential vanilloid 1 receptor (TRPV1) is a non-selective cation channel that is widely expressed in the peripheral nervous system and has been found to be activated by the eCB anandamide (Ross, 2003). In the central nervous system, expression of TRPV1 and its functional significance have been observed in various brain regions (Brown, Chirila, Schrank, & Kauer, 2013; Gibson, Edwards, Page, Van Hook, & Kauer, 2008; Kauer & Gibson, 2009; Marinelli, Pascucci, Bernardi, Puglisi-Allegra, & Mercuri, 2005; Musella et al., 2009; Zschenderlein, Gebhardt, von Bohlen Und Halbach, Kulisch, & Albrecht, 2011). Similar to NMDAR-dependent LTD, eCB-mediated TRPV1-dependent LTD results in the removal of postsynaptic AMPARs and has been observed in both the NAc core (Grueter, Brasnjo, & Malenka, 2010) and the NAc shell (Renteria, Jeanes, & Morrisett, 2014).

3. EVIDENCE IMPLICATING NMDAR-DEPENDENT POSTSYNAPTIC AMPAR ENDOCYTOSIS (LTD) IN RESPONSE TO DRUGS OF ABUSE

3.1 Cocaine and Other Psychostimulants

Drug-induced disruption of NMDAR-dependent LTD in the NAc was first described by Thomas and colleagues in 2001 (Thomas, Beurrier, Bonci, & Malenka, 2001). Daily intraperitoneal injections of cocaine resulted in the sensitization of the locomotor response to cocaine. In brain slices taken from mice that show behavioral sensitization, there was a decrease in the AMPA/NMDA ratio and a decrease in AMPAR-mediated miniature excitatory postsynaptic current (mEPSC) amplitude, suggesting a decrease in the number of postsynaptic AMPARs present at the synapse. The cocaine-induced internalization of AMPARs resulted in an occlusion of the expression of NMDAR-dependent LTD. It was later shown that the effects of cocaine on NAc plasticity are dependent on the history of cocaine exposure. Mice exhibiting an occlusion of LTD expression were treated with daily injections of cocaine but did not receive a challenge dose to test for behavioral sensitization (Kourrich, Rothwell, Klug, & Thomas, 2007). Instead, mice in extended cocaine withdrawal showed an increase in AMPA/NMDA ratio and an increase in mEPSC amplitude. These findings suggest an increase in AMPAR signaling following only extended withdrawal from cocaine exposure.

Other groups have shown similar alterations in AMPAR-mediated signaling in response to amphetamine exposure in which the drug-induced internalization of GluA2-containing AMPARs may be necessary for the expression of amphetamine locomotor sensitization (Brebner et al., 2005; Choi, Ahn, Wang, & Phillips, 2014). Sensitization to amphetamine treatment was blocked by the use of the HIV-1 TAT protein to deliver a peptide that mimics the C-terminus of the GluA2 subunit, which occludes GluA2 subunit-containing AMPAR internalization. Application of this peptide directly into the NAc abolished stereotypies that had developed with psychomotor sensitization. However, these stereotypies remained unchanged when the peptide was injected into the VTA. These findings are significant in that it is one of the first examples of altering a drug-induced phenotype by disrupting the drug-induced changes in excitatory signaling in the NAc.

The importance of the expression of a drug-induced LTD-like state is further supported by a study of cocaine self-administration in rats (Kasanetz et al., 2010). In rats that self-administered cocaine, only a subset of animals showed the persistent drug-seeking characteristics similar to those seen in human cocaine dependents (Deroche-Gamonet, Belin, & Piazza, 2004). Rats were separated into groups termed “addicted” or “nonaddicted” based on their score from three addiction-like behaviors paralleling those defined by the DSM-IV. Two weeks after the last self-administration session, LTD was impaired in all animals but gradually recovered in “nonaddicts.” In “addicts,” LTD was persistently impaired which suggests that the long-lasting impairment of LTD may be important for the transition from drug seeking to dependence.

3.2 Ethanol

Ethanol is a potent modulator of plasticity. Both, in vitro and in vivo exposure to ethanol can disrupt the expression of synaptic plasticity (McCool, 2011; Morrisett & Swartzwelder, 1993). Work from our laboratory has shown that in vitro ethanol can inhibit NMDARs in the NAc (Malvae et al., 2002; Zhang, Hendricson, & Morrisett, 2005) and block the expression of NMDAR-dependent LTD, while chronic intermittent in vivo exposure results in a reversal in the expression of plasticity (Jeanes, Buske, & Morrisett, 2011). Chronic intermittent ethanol exposure (CIE) is a widely accepted model used to induce ethanol dependence in C57Bl/6 mice (Becker & Lopez, 2004). Mice are exposed to ethanol vapor during four consecutive daily 16-h sessions to elicit intoxication (target blood ethanol concentrations (BECs) of approximately 40–50 mM with coadministration of pyrazole). This protocol is normally repeated weekly two to four times, and elevates two-bottle choice (2BC) ethanol preference and intake by approximately 50%. Twenty-four hours after CIE, the same induction protocol that results in LTD in ethanol-naïve mice will induce the expression of LTP in brain slices from ethanol-exposed C57Bl/6J mice. The disruption of NMDAR-dependent LTD persists for up to 72 h into withdrawal from ethanol vapor and results in the absence of both, LTD and LTP. Interestingly, in the hippocampal formation, we previously reported a lack of ethanol inhibition of low-frequency NMDAR-dependent LTD suggesting significant differences in the mechanisms mediating this form of plasticity between hippocampal and accumbal brain regions (Hendricson, Miao, Lippmann, & Morrisett, 2002).

Behavioral sensitization to ethanol also alters NMDAR-dependent NAc LTD in mice. In Swiss Webster mice treated with daily ethanol injections, only a portion showed locomotor sensitization, and only this portion displayed a disruption of NMDAR-dependent accumbal LTD (Abrahao et al., 2013). This effect was the result of dampened NMDAR function as Western blot analysis showed a measured decrease of the NR1 subunit. In another study, the expression of NMDAR-dependent LTD was found to be hampered in the NAc of ethanol-dependent rats. In rats consuming an ethanol-containing diet for 20 days, alterations in plasticity and MSN morphology were observed 12 h into ethanol withdrawal. The ethanol-containing diet led to the loss of long thin dendritic spines as well as a decrease in NMDAR function (Spiga et al., 2014).

4. LENTIVIRAL OCCLUSION OF GluA2 SUBUNIT INTERNALIZATION MODULATES CIE-ENHANCED ETHANOL INTAKE

Given the importance of neuroadaptations of AMPARs in the NAc for the expression of drug-induced behaviors, the use of the synthetic peptide “GluA2_{3Y}” derived from the rat GluA2 carboxyl tail (⁸⁶⁹YKEGYNVY⁸⁷⁷) could prove to be effective for the attenuation of ethanol-induced behaviors as well. As previously described, Wang and colleagues’ (Brebner et al., 2005) GluA2_{3Y} peptide saturates the cellular machinery that binds to GluA2 and thus prevents the clathrin-mediated internalization of AMPARs and the subsequent formation of LTD (see Fig. 1). To study the effect of LTD occlusion in chronically ethanol-consuming animals, we designed a virus that causes long-term over-expression of the GluA2_{3Y} peptide. As predicted, NAc MSNs of animals injected with the virus overexpressing the GluA2_{3Y} peptide showed an occlusion of LTD in slice (Maier et al., 2015). However, this change in synaptic plasticity did not alter ethanol drinking in animal models of moderate ethanol consumption (ie, operant self-administration and 2BC). Interestingly, CIE exposure, a model that leads to prolonged high BECs resulting in increased ethanol consumption, did not affect drinking in mice injected with the GluA2_{3Y} peptide. These findings support the notion that AMPAR trafficking plays an important role in alcohol reinforcement during intense levels of ethanol exposure.

5. DIFFERENTIAL INVOLVEMENT OF D1 DOPAMINE RECEPTOR-EXPRESSING VS D2 DOPAMINE RECEPTOR-EXPRESSING MEDIUM SPINY NEURONS OF THE SHELL OF THE NUCLEUS ACCUMBENS IN NMDAR-LTD

In both the core and shell subregions of the NAc, there are two major subtypes of MSNs depending on the dopamine receptor expression. Medium spiny neurons that express the D1 dopamine receptor coexpress the signaling peptides dynorphin and substance P (D1 MSN), while MSNs that express the D2 dopamine receptor also express enkephalin (D2 MSN) (Lu, Ghasemzadeh, & Kalivas, 1998; Zhou, Furuta, & Kaneko, 2003). D1 and D2 MSNs of the NAc have similar projection patterns to the direct and indirect pathways of the dorsal striatum although the segregation of pathways is not as clearly distinct, and therefore that terminology is no longer applied to the accumbal projections (Humphries & Prescott, 2010; Kupchik et al., 2015; Sesack & Grace, 2010; Smith, Lobo, Spencer, & Kalivas, 2013). In the

NAc core and shell, D1 MSNs project to the substantia nigra pars reticulata and the VTA, respectively, whereas shell D1 MSNs also project to the ventral pallidum. D2 MSNs from the core and shell project primarily to the ventral pallidum.

The development of transgenic mice that express a fluorophore (such as enhanced green fluorescent protein (eGFP) or tdTomato) under the control of either the D1 or D2 dopamine receptor promoter (Matamales et al., 2009; Valjent, Bertran-Gonzalez, Herve, Fisone, & Girault, 2009) has allowed for greater insight as to how these two populations of MSNs differ. In the dorsal striatum, the electrophysiological properties of D1 MSNs differ from those of D2 MSNs (Cepeda et al., 2008; Day, Wokosin, Plotkin, Tian, & Surmeier, 2008; Gertler, Chan, & Surmeier, 2008). Similarly in the NAc core, D1 and D2 MSNs have different electrophysiological and synaptic properties (Grueter et al., 2010). Given these differences in basal electro-physiological properties, it might also be expected that D1 and D2 MSNs would display differences in the expression of plasticity. Work from our laboratory using *Drd1a*-eGFP transgenic mice confirms the differential expression of plasticity such that conventional LTD conditioning stimuli elicit NMDAR-dependent LTD only in the shell D1 MSNs and not in D1-lacking MSNs (Jeanes et al., 2014).

6. CHRONIC INTERMITTENT ETHANOL EXPOSURE DIFFERENTIALLY MODULATES D1 VS D2 MSN PLASTICITY

CIE exposure is a well-established model for inducing ethanol dependence and increasing volitional ethanol intake in mice (Becker & Lopez, 2004; Griffin, Lopez, & Becker, 2009; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Griffin, 2014; Lopez & Becker, 2005). As described above, we have previously reported that CIE vapor exposure disrupts NMDAR-dependent LTD in the NAc shell of C57Bl/6J mice (Jeanes et al., 2011). For this initial characterization of ethanol-induced metaplasticity in wild-type C57Bl/6J mice, we did not have the ability to selectively record from the D1 or D2 MSN subtypes. To determine if ethanol exposure differentially modulates plasticity in D1 or D2 MSNs we used *Drd1a*-eGFP transgenic mice on a Swiss Webster background (Jeanes et al., 2014). First, we found that NMDAR-dependent LTD was expressed only in eGFP-positive (presumed D1) MSNs in the NAc shell and that the LTD conditioning protocol had no long-term effect on EPSC amplitudes in eGFP-negative (presumed D2) MSNs. Second, 24 h after CIE, there was a reversal in the expression of plasticity; the LTD protocol resulted in LTD in D2 MSNs and had no effect on D1 MSNs. Over a 2-week period, the expression of plasticity is gradually restored in D1 MSNs and abolished in D2 MSNs.

Given the discrepancy in the polarity of plasticity after ethanol vapor exposure between C57Bl/6J and *Drd1a*-eGFP on the Swiss Webster background, the ethanol-induced alterations of plasticity seem to be specific to the strain of mouse. The CIE-induced increase in volitional ethanol intake has been characterized primarily in C57Bl/6J mice. Interpretation of our data collected from Swiss Webster mice may be limited by the fact that the latter strain does not voluntarily drink significant amounts of ethanol. To investigate how CIE modulates plasticity in D1 and D2 MSNs of a mouse strain that has a well-documented drinking phenotype, we used *Drd1a*-tdTomato transgenic mice on a C57Bl/6J background

(Ade, Wan, Chen, Gloss, & Calakos, 2011). Similar to what we observed in transgenic mice on the Swiss Webster background, LTD was expressed only in D1 MSNs of ethanol-naïve mice (Renteria, Maier, Buske, & Morrisett, 2016). Twenty-four hours after CIE treatment, the pairing protocol resulted in LTP in D1 MSNs and LTD in D2 MSNs. This alteration in the expression of plasticity was accompanied by a change in NMDAR function, measured using NMDA/AMPA ratios as well as an input output curve of isolated NMDAR currents. We found an increase in NMDAR function in D1MSNs and a decrease in D2 MSNs. These findings are illustrated in Fig. 2.

7. STRATEGY FOR TARGET VALIDATION USING ACCUMBAL PLASTICITY

A variety of screening measures can frequently implicate particular genes or proteins in drug-related behaviors or experience. Determining whether such novel targets might indeed develop into fruitful avenues of therapeutic development is a very daunting task. Nevertheless, we and others have observed very consistent plasticity changes in the NAc across different drugs of abuse as well as between different mouse strains and even cell-specific changes across these different mouse lines. We feel that such consistent observations strongly implicate accumbal plasticity as an important neuroadaptive process underlying drug-induced behaviors, particularly in the early developmental stages of drug seeking. Therefore, we envision that when modulation of a particular target alters accumbal plasticity in NAc D1 MSNs, such a target would constitute an especially strong candidate for further investigation. Herein, we summarize our work in this area in Table 1, and describe in greater detail in the following sections two examples of such an approach focusing on targets identified by a number of laboratories from animal models of excessive ethanol consumption.

7.1 Studies on the HDID and HS/Npt Mouse Lines

Drinking in the dark (DID) is a rodent model of binge ethanol consumption. A single ethanol tube (20% (v/v)) is inserted in the home cage 3 h into the dark cycle for 2 h for 3 consecutive days, followed by a 4-h exposure on the fourth day. This model has been shown to produce intoxicating levels of consumption in mice (Rhodes, Best, Belknap, Finn, & Crabbe, 2005). Crabbe et al. (2009) developed an outbred strain termed HS/Npt from systematic crossings of multiple inbred strains. HS/Npt mice underwent DID procedures and those mice with the highest BECs following DID were selectively bred together, producing mice termed high drinking in the dark (HDID) mice. By the eleventh generation the HDID BECs and ethanol consumption, in the DID paradigm, were significantly higher than that of the progenitor HS/Npt mice (Crabbe et al., 2009).

Subsequent testing of the HDID mice in comparison to the progenitor HS/Npt mice indicated that HDID mice exhibit increased sensitivity to ethanol. Later generations of HDID mice retained greater BECs compared to HS/Npts following DID, as well as consumed modestly more ethanol in a limited access 2BC paradigm. HDID mice, however, do not consume greater quantities of ethanol than HS/Npt mice during a 24-h 2BC drinking paradigm. These findings indicate that selective breeding for high BECs selects for alleles distinct from those that confer high consumption in continuous access preference paradigms

(Crabbe, Spence, Brown, & Metten, 2011). Regarding differences in drinking, HDID mice show greater ethanol-stimulated locomotor activity and greater sensitivity to some but not all ethanol-sensitive behavioral tasks as compared to HS/Npt mice (Crabbe et al., 2012). These findings indicate that some ethanol responses share common genetic control with the ability to reach high BEC after DID procedures. The HDID mice also exhibit a decreased sensitivity to ethanol-induced hypothermia compared to the HS/Npts (Crabbe et al., 2012).

As previously discussed in this chapter, we maintain that alterations in synaptic plasticity within the NAc following ethanol exposure are critical for the expression of ethanol-related behaviors. Since there is a strong difference in ethanol consumption phenotypes, we hypothesized that a difference in synaptic plasticity would be observed in NAc shell D1 MSNs between the HDID and HS/Npt mice. Thus, whole-cell patch clamp recordings were conducted on putative D1 MSNs identified by injection of a fluorescent tracer (cholera toxin subunit B) into the VTA to retrogradely label VTA-projecting D1 MSNs from the NAc shell. For ethanol-naïve HDID and HS/Npt mice, no differences were observed in LTD induction between the two lines (see Table 1). We then examined if these mice exhibit differential LTD expression 24 h after a 4-day bout of CIE exposure. The HDID mice exhibited a loss of LTD similar to what had been observed in C57Bl/6J mice (Jeanes et al., 2011, 2014; Renteria et al., under revision). Interestingly, the HS/Npt mice did not exhibit a reduction in LTD magnitude following CIE. We are currently conducting more experiments to better understand differences in accumbal synaptic plasticity relative to ethanol exposure between these two lines of mice. Nevertheless, these findings suggest that selective breeding for high BECs does not impact accumbal plasticity or CIE-induced metaplasticity, but the converse is not true such that following CIE HS/Npt mice display an electrophysiological phenotype unique from mice that drink greater amounts of ethanol (Jeanes et al., 2011, 2014; Renteria et al., under revision).

7.2 Studies Concerning the Receptor Tyrosine Kinase ALK

Another molecular target that we have screened for its role in accumbal synaptic plasticity is anaplastic lymphoma kinase (ALK). ALK is a receptor tyrosine kinase that was discovered and initially characterized for its role in non-Hodgkin's, anaplastic large cell lymphoma (Iwahara et al., 1997; Morris et al., 1994, 1997). These early studies of ALK found its mRNA and protein to be located specifically in neural tissues of mice and humans, with expression highest during embryogenesis then declining postnatally until reaching and maintaining a low level of expression into adulthood (Iwahara et al., 1997; Morris et al., 1997; Pulford et al., 1997). This spatio-temporal pattern of expression pointed toward a role for ALK in normal nervous system development, which was supported by subsequent investigations of ALK in a number of species (Degoutin, Brunet-de Carvalho, Cifuentes-Diaz, & Vigny, 2009; Hurley, Clary, Copie, & Lefcort, 2006; Liao, Hung, Abrams, & Zhen, 2004; Rohrbough & Broadie, 2010; Yao et al., 2013). *Alk* transcription is regulated by LIM domain-only (LMO) proteins, which interact with DNA-binding elements to repress *Alk* transcription (Lasek, Gesch, Giorgetti, Kharazia, & Heberlein, 2011; Lasek, Lim, et al., 2011; Lasek, Giorgetti, Berger, Taylor, & Heberlein, 2011), and it is this relationship that brought ALK to the attention of alcohol researchers.

The laboratory of Ulrike Heberlein has long championed *Drosophila melanogaster* (the fruit fly) as a model organism to screen for gene mutations that alter responses to drugs and alcohol (Devineni et al., 2011; Heberlein, Tsai, Kapfhamer, & Lasek, 2009). Using such genetic screening methods, mutations in the fly gene for LMO that affect behavioral sensitivity to cocaine were identified (Tsai, Bainton, Blau, & Heberlein, 2004). In a subsequent series of elegant papers, Lasek and colleagues reported that LMO proteins also affect responses to cocaine in mice, and to ethanol in both flies and mice (Lasek, Giorgetti, et al., 2011; Lasek et al., 2010; Savarese, Zou, Kharazia, Maiya, & Lasek, 2014). ALK was then identified as a transcriptional target of LMO proteins which might mediate the observed effects of cocaine and ethanol (Lasek, Gesch, et al., 2011; Lasek, Lim, et al., 2011); *Alk* mRNA expression in the striatum is inversely correlated with ethanol consumption in the BXD line of inbred mice, and mice with a genetic deletion in a portion of the *Alk* coding sequence (ALK KO) drink more ethanol relative to ALK WT mice when tested in the DID paradigm. More recently, the Lasek group has identified how ethanol affects ALK activity and ALK-regulated intracellular signaling cascades. In both cell culture and mouse brain, ethanol treatment activates ALK and ERK signaling, as evidenced by increased phosphorylation of these proteins (He, Chen, Muramatsu, & Lasek, 2015). Moreover, pretreatment with an inhibitor of ALK, TAE684 (Galkin et al., 2007), prevents the ethanol-induced activation of ERK. As ERK has been shown by several groups to modulate ethanol-related behaviors (Agoglia et al., 2015; Faccidomo, Besheer, Stanford, & Hodge, 2009; Faccidomo, Salling, Galunas, & Hodge, 2015), these latest findings point toward a possible biochemical mechanism for ALK's influence on ethanol consumption.

To summarize the findings from these cell and animal models, preexisting variations in *Alk* expression influence ethanol consumption and associated behavioral sensitivity, while ethanol acutely activates ALK and ALK-dependent signaling pathways that influence ethanol-related behaviors. Thus, given that ALK–ethanol interactions play a role in regulating ethanol consumption alongside the previously discussed relationship between NMDAR-dependent LTD and ethanol experience, our lab has collaborated with Dr. Lasek to investigate the potential involvement of ALK in the induction of LTD by NAc shell D1 MSNs (Mangieri & Morrisett, 2015). In order to specifically examine plasticity induction in D1 MSNs, we crossed the ALK KO line of mice with *Drd1a*-tdTomato mice (Ade et al., 2011) to generate ALK WT and ALK KO mice that were also hemizygous for *Drd1a*-tdTomato. When we compared the magnitude of depression induced in D1 MSNs of each genotype, we found LTD to be markedly reduced, but not absent, in the ALK KO mice. To determine if acute, pharmacological inhibition of ALK activity would similarly affect LTD induction in *Drd1a*-tdTomato mice, we pretreated brain slices with TAE684, and observed a concentration-dependent attenuation of LTD. The findings of the plasticity experiments are summarized in Table 1. We also recorded spontaneous excitatory postsynaptic currents in D1 MSNs, and found that the event amplitude, but not frequency, was significantly elevated for ALK KO mice and ALK WT slices pretreated with TAE684. All together, these observations suggest that one function of ALK in the adult mouse brain may be to regulate postsynaptic AMPAR trafficking.

In conclusion, the combined genetic, biochemical, electrophysiological, and behavioral approaches to studying ALK in model systems have highlighted it as a target needing further

investigation for the development of novel drugs to treat alcohol use disorders. Indeed, there are at least two reports of findings in human subjects that support this idea. One, from the Lasek group, examined the *ALK* gene sequence in humans and discovered two single-nucleotide polymorphisms (SNPs) in the coding sequence that were significantly associated with responses to ethanol (subjective high and lateral body sway) by subjects in a laboratory setting (Lasek, Lim, et al., 2011). The second report, by a different group, performed a meta-analysis of two genome-wide association studies and found strong association between an *ALK* SNP and alcohol dependence in humans (Wang et al., 2011). Although it is unknown as to whether any of these SNPs confer differences in ALK function, these findings nevertheless are consistent with the idea that ALK is a promising target for modulating responses to ethanol in humans. Collaborative experiments are ongoing between our lab and that of Dr. Lasek's to further validate and explore ALK as a target for modulating effects of ethanol in the brain.

8. CONCLUSIONS

In this review, nine experiments were chosen based on either an animal model of enhanced ethanol intake or sensitivity, or on some type of screening mechanism that identified potential targets for medication development. As documented in Table 1, we have investigated the expression of baseline plasticity or its alteration following some type of manipulation. In multiple lines of mice, we have consistently observed that NMDAR-dependent LTD of glutamatergic synaptic transmission is differentially expressed between D1 and D2 MSNs of the NAc shell. This form of synaptic plasticity is highly sensitive to intoxicating concentrations of ethanol applied acutely to these neurons and is markedly altered (ie, metaplastic) following CIE exposure. We have tested three different targets (ALK, CD14, and BK β 1 subunit), implicated by numerous other laboratories using some ethanol-relevant screening protocol, for alterations in accumbal plasticity, but we only observed very prominent and consistent effects following genetic and pharmacological manipulation of ALK. Moreover, work on ALK continues to be a major focus of our laboratory, and we are preparing to investigate the effects of FDA-approved ALK inhibitors on this metaplasticity and ethanol drinking as well. Therefore, taken together, our findings suggest that there is significant predictive value in accumbal plasticity studies to screen and validate the repurposing of medications to treat alcohol abuse and dependence.

REFERENCES

- Abraham KP, Ariwodola OJ, Butler TR, Rau AR, Skelly MJ, Carter E, et al. (2013). Locomotor sensitization to ethanol impairs NMDA receptor-dependent synaptic plasticity in the nucleus accumbens and increases ethanol self-administration. *The Journal of Neuroscience*, 33(11), 4834–4842. 10.1523/JNEUROSCI.583911.2013. [PubMed: 23486954]
- Ade KK, Wan Y, Chen M, Gloss B, & Calakos N (2011). An improved BAC transgenic fluorescent reporter line for sensitive and specific identification of striatonigral medium spiny neurons. *Frontiers in Systems Neuroscience*, 5, 32 10.3389/fnsys.2011.00032. [PubMed: 21713123]
- Agoglia AE, Sharko AC, Psilos KE, Holstein SE, Reid GT, & Hodge CW (2015). Alcohol alters the activation of ERK1/2, a functional regulator of binge alcohol drinking in adult C57BL/6J mice. *Alcoholism, Clinical and Experimental Research*, 39(3), 463–475. 10.1111/acer.12645.

- Ambroggi F, Ishikawa A, Fields HL, & Nicola SM (2008). Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron*, 59(4), 648–661. 10.1016/j.neuron.2008.07.004. [PubMed: 18760700]
- Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, et al. (2000). Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nature Neuroscience*, 3(12), 1291–1300. 10.1038/81823. [PubMed: 11100150]
- Becker HC, & Lopez MF (2004). Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. *Alcoholism, Clinical and Experimental Research*, 28(12), 1829–1838.
- Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, et al. (2005). Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science*, 310(5752), 1340–1343. 10.1126/science.1116894. [PubMed: 16311338]
- Brown TE, Chirila AM, Schrank BR, & Kauer JA (2013). Loss of interneuron LTD and attenuated pyramidal cell LTP in Trpv1 and Trpv3 KO mice. *Hippocampus*, 23(8), 662–671. 10.1002/hipo.22125. [PubMed: 23536486]
- Carlezon WA, Jr., Devine DP, & Wise RA (1995). Habit-forming actions of nomifensine in nucleus accumbens. *Psychopharmacology*, 122(2), 194–197. 10.1007/BF02246095. [PubMed: 8848536]
- Cepeda C, Andre VM, Yamazaki I, Wu N, Kleiman-Weiner M, & Levine MS (2008). Differential electrophysiological properties of dopamine D1 and D2 receptor-containing striatal medium-sized spiny neurons. *The European Journal of Neuroscience*, 27(3), 671–682. 10.1111/j.1460-9568.2008.06038.x. [PubMed: 18279319]
- Choi FY, Ahn S, Wang YT, & Phillips AG (2014). Interference with AMPA receptor endocytosis: Effects on behavioural and neurochemical correlates of amphetamine sensitization in male rats. *Journal of Psychiatry & Neuroscience*, 39(3), 189–199. [PubMed: 24290077]
- Crabbe JC, Kruse LC, Colville AM, Cameron AJ, Spence SE, Schlumbohm JP, et al. (2012). Ethanol sensitivity in high drinking in the dark selectively bred mice. *Alcoholism: Clinical and Experimental Research*, 36(7), 1162–1170. 10.1111/j.1530-0277.2012.01735.x.
- Crabbe JC, Metten P, Rhodes JS, Yu CH, Brown LL, Phillips TJ, et al. (2009). A line of mice selected for high blood ethanol concentrations shows drinking in the dark to intoxication. *Biological Psychiatry*, 65(8), 662–670. 10.1016/j.biopsych.2008.11.002. [PubMed: 19095222]
- Crabbe JC, Spence SE, Brown LL, & Metten P (2011). Alcohol preference drinking in a mouse line selectively bred for high drinking in the dark. *Alcohol*, 45(5), 427–440. 10.1016/j.alcohol.2010.12.001. [PubMed: 21194877]
- Day M, Wokosin D, Plotkin JL, Tian X, & Surmeier DJ (2008). Differential excitability and modulation of striatal medium spiny neuron dendrites. *The Journal of Neuroscience*, 28(45), 11603–11614. 10.1523/JNEUROSCI.1840-08.2008. [PubMed: 18987196]
- Degoutin J, Brunet-de Carvalho N, Cifuentes-Diaz C, & Vigny M (2009). ALK (Anaplastic Lymphoma Kinase) expression in DRG neurons and its involvement in neuron-Schwann cells interaction. *The European Journal of Neuroscience*, 29(2), 275–286. 10.1111/j.1460-9568.2008.06593.x. [PubMed: 19200234]
- Deroche-Gamonet V, Belin D, & Piazza PV (2004). Evidence for addiction-like behavior in the rat. *Science*, 305(5686), 1014–1017. 10.1126/science.1099020. [PubMed: 15310906]
- Devineni AV, McClure KD, Guarnieri DJ, Corl AB, Wolf FW, Eddison M, et al. (2011). The genetic relationships between ethanol preference, acute ethanol sensitivity, and ethanol tolerance in *Drosophila melanogaster*. *Fly*, 5(3), 191–199. [PubMed: 21750412]
- Di Chiara G (2002). Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. *Behavioural Brain Research*, 137(1–2), 75–114. 10.1016/S0166-4328(02)00286-3. [PubMed: 12445717]
- Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, et al. (2004). Dopamine and drug addiction: The nucleus accumbens shell connection. *Neuropharmacology*, 47(Suppl. 1), 227–241. 10.1016/j.neuropharm.2004.06.032. [PubMed: 15464140]
- Di Chiara G, & Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the*

- National Academy of Sciences of the United States of America, 85(14), 5274–5278. [PubMed: 2899326]
- Doyon WM, York JL, Diaz LM, Samson HH, Czachowski CL, & Gonzales RA (2003). Dopamine activity in the nucleus accumbens during consummatory phases of oral ethanol self-administration. *Alcoholism, Clinical and Experimental Research*, 27(10), 1573–1582. 10.1097/01.ALC.0000089959.66222.B8.
- Faccidomo S, Besheer J, Stanford PC, & Hodge CW (2009). Increased operant responding for ethanol in male C57BL/6J mice: Specific regulation by the ERK1/2, but not JNK, MAP kinase pathway. *Psychopharmacology*, 204(1), 135–147. 10.1007/s00213-008-1444-9. [PubMed: 19125235]
- Faccidomo S, Salling MC, Galunas C, & Hodge CW (2015). Operant ethanol self-administration increases extracellular-signal regulated protein kinase (ERK) phosphorylation in reward-related brain regions: Selective regulation of positive reinforcement in the prefrontal cortex of C57BL/6J mice. *Psychopharmacology*, 232(18), 3417–3430. 10.1007/s00213-015-3993-z. [PubMed: 26123321]
- Fiorillo CD, Tobler PN, & Schultz W (2003). Discrete coding of reward probability and uncertainty by dopamine neurons. *Science*, 299, 1898–1902. 10.1126/science.1077349. [PubMed: 12649484]
- Galkin AV, Melnick JS, Kim S, Hood TL, Li N, Li L, et al. (2007). Identification of NVP-TAE684, a potent, selective, and efficacious inhibitor of NPM-ALK. *Proceedings of the National Academy of Sciences of the United States of America*, 104(1), 270–275. 10.1073/pnas.0609412103. [PubMed: 17185414]
- Gertler TS, Chan CS, & Surmeier DJ (2008). Dichotomous anatomical properties of adult striatal medium spiny neurons. *The Journal of Neuroscience*, 28(43), 10814–10824. 10.1523/JNEUROSCI.2660-08.2008. [PubMed: 18945889]
- Gibson HE, Edwards JG, Page RS, Van Hook MJ, & Kauer JA (2008). TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. *Neuron*, 57(5), 746–759. 10.1016/j.neuron.2007.12.027. [PubMed: 18341994]
- Griffin WC (2014). Alcohol dependence and free-choice drinking in mice. *Alcohol (Fayetteville, NY)*, 48(3), 287–293. 10.1016/j.alcohol.2013.11.006.
- Griffin WC, 3rd., Lopez MF, & Becker HC (2009a). Intensity and duration of chronic ethanol exposure is critical for subsequent escalation of voluntary ethanol drinking in mice. *Alcoholism, Clinical and Experimental Research*, 33(11), 1893–1900. 10.1111/j.1530-0277.2009.01027.x.
- Griffin WC, 3rd., Lopez MF, Yanke AB, Middaugh LD, & Becker HC (2009b). Repeated cycles of chronic intermittent ethanol exposure in mice increases voluntary ethanol drinking and ethanol concentrations in the nucleus accumbens. *Psychopharmacology*, 201(4), 569–580. 10.1007/s00213-008-1324-3. [PubMed: 18791704]
- Gruber AJ, Hussain RJ, & O'Donnell P (2009). The nucleus accumbens: A switchboard for goal-directed behaviors. *PloS One*, 4(4), e5062 10.1371/journal.pone.0005062. [PubMed: 19352511]
- Grueter BA, Brasnjo G, & Malenka RC (2010). Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nature Neuroscience*, 13(12), 1519–1525. 10.1038/nn.2685. [PubMed: 21076424]
- Haber SN, Fudge JL, & McFarland NR (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *The Journal of Neuroscience*, 20(6), 2369–2382. [PubMed: 10704511]
- He D, Chen H, Muramatsu H, & Lasek AW (2015). Ethanol activates midline and anaplastic lymphoma kinase signaling in neuroblastoma cells and in the brain. *Journal of Neurochemistry*, 135(3), 508–521. 10.1111/jnc.13252. [PubMed: 26206265]
- Heberlein U, Tsai LT-Y, Kapfhammer D, & Lasek AW (2009). *Drosophila*, a genetic model system to study cocaine-related behaviors: A review with focus on LIM-only proteins. *Neuropharmacology*, 56(Suppl. 1), 97–106. 10.1016/j.neuropharm.2008.07.023. [PubMed: 18694769]
- Heifets BD, & Castillo PE (2009). Endocannabinoid signaling and long-term synaptic plasticity. *Annual Review of Physiology*, 71, 283–306. 10.1146/annurev.physiol.010908.163149.
- Hendricson AW, Miao CLA, Lippmann MJ, & Morrisett RA (2002). Ifenprodil and ethanol enhance NMDA receptor-dependent long-term depression. *The Journal of Pharmacology and Experimental Therapeutics*, 301(3), 938–944. [PubMed: 12023522]

- Hoffman AF, Oz M, Caulder T, & Lupica CR (2003). Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *The Journal of Neuroscience*, 23(12), 4815–4820. doi: 0270-6474/03/234815-06 [pii]. [PubMed: 12832502]
- Humphries MD, & Prescott TJ (2010). The ventral basal ganglia, a selection mechanism at the crossroads of space, strategy, and reward. *Progress in Neurobiology*, 90(4), 385–417. 10.1016/j.pneurobio.2009.11.003. [PubMed: 19941931]
- Hurley SP, Clary DO, Copie V, & Lefcort F (2006). Anaplastic lymphoma kinase is dynamically expressed on subsets of motor neurons and in the peripheral nervous system. *The Journal of Comparative Neurology*, 495(2), 202–212. 10.1002/cne.20887. [PubMed: 16435287]
- Ikemoto S (2007). Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Research Reviews*, 56(1), 27–78. 10.1016/j.brainresrev.2007.05.004. [PubMed: 17574681]
- Imperato A, & Di Chiara G (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *The Journal of Pharmacology and Experimental Therapeutics*, 239(1), 219–228. [PubMed: 3761194]
- Ito R, Robbins TW, Pennartz CM, & Everitt BJ (2008). Functional interaction between the hippocampus and nucleus accumbens shell is necessary for the acquisition of appetitive spatial context conditioning. *The Journal of Neuroscience*, 28(27), 6950–6959. 10.1523/JNEUROSCI.1615-08.2008. [PubMed: 18596169]
- Iwahara T, Fujimoto J, Wen D, Cupples R, Bucay N, Arakawa T, et al. (1997). Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene*, 14(4), 439–449. 10.1038/sj.onc.1200849. [PubMed: 9053841]
- Jeanes ZM, Buske TR, & Morrisett RA (2011). In vivo chronic intermittent ethanol exposure reverses the polarity of synaptic plasticity in the nucleus accumbens shell. *The Journal of Pharmacology and Experimental Therapeutics*, 336(1), 155–164. 10.1124/jpet.110.171009. [PubMed: 20947635]
- Jeanes ZM, Buske TR, & Morrisett RA (2014). Cell type-specific synaptic encoding of ethanol exposure in the nucleus accumbens shell. *Neuroscience*, 277, 184–195. 10.1016/j.neuroscience.2014.06.063. [PubMed: 25003712]
- Kalivas PW, Volkow N, & Seamans J (2005). Unmanageable motivation in addiction: A pathology in prefrontal-accumbens glutamate transmission. *Neuron*, 45(5), 647–650. 10.1016/j.neuron.2005.02.005. [PubMed: 15748840]
- Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, et al. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science*, 328(5986), 1709–1712. 10.1126/science.1187801. [PubMed: 20576893]
- Kauer JA, & Gibson HE (2009). Hot flash: TRPV channels in the brain. *Trends in Neurosciences*, 32(4), 215–224. 10.1016/j.tins.2008.12.006. [PubMed: 19285736]
- Kombian SB, & Malenka RC (1994). Simultaneous LTP of non-NMDA- and LTD of NMDA-receptor-mediated responses in the nucleus accumbens. *Nature*, 368(6468), 242–246. 10.1038/368242a0. [PubMed: 7908412]
- Kourrich S, Rothwell PE, Klug JR, & Thomas MJ (2007). Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *The Journal of Neuroscience*, 27(30), 7921–7928. 10.1523/JNEUROSCI.1859-07.2007. [PubMed: 17652583]
- Kupchik YM, Brown RM, Heinsbroek JA, Lobo MK, Schwartz DJ, & Kalivas PW (2015). Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nature Neuroscience*, 18(9), 1230–1232. 10.1038/nn.4068. [PubMed: 26214370]
- Lasek AW, Gesch J, Giorgetti F, Kharazia V, & Heberlein U (2011). Alk is a transcriptional target of LMO4 and ERα that promotes cocaine sensitization and reward. *The Journal of Neuroscience*, 31(40), 14134–14141. 10.1523/JNEUROSCI.3415-11.2011. [PubMed: 21976498]
- Lasek AW, Giorgetti F, Berger KH, Taylor S, & Heberlein U (2011). Lmo genes regulate behavioral responses to ethanol in *Drosophila melanogaster* and the mouse. *Alcoholism, Clinical and Experimental Research*, 35(9), 1600–1606. 10.1111/j.1530-0277.2011.01506.x.
- Lasek AW, Kapfhamer D, Kharazia V, Gesch J, Giorgetti F, & Heberlein U (2010). Lmo4 in the nucleus accumbens regulates cocaine sensitivity. *Genes, Brain, and Behavior*, 9(7), 817–824. 10.1111/j.1601-183X.2010.00620.x.

- Lasek AW, Lim J, Kliethermes CL, Berger KH, Joslyn G, Brush G, et al. (2011). An evolutionary conserved role for anaplastic lymphoma kinase in behavioral responses to ethanol. *PloS One*, 6(7), e22636 10.1371/journal.pone.0022636.
- Li Y, & Kauer JA (2004). Repeated exposure to amphetamine disrupts dopaminergic modulation of excitatory synaptic plasticity and neurotransmission in nucleus accumbens. *Synapse (New York)*, 51(1), 1–10. 10.1002/syn.10270.
- Liao EH, Hung W, Abrams B, & Zhen M (2004). An SCF-like ubiquitin ligase complex that controls presynaptic differentiation. *Nature*, 430(6997), 345–350. 10.1038/nature02647. [PubMed: 15208641]
- Lopez MF, & Becker HC (2005). Effect of pattern and number of chronic ethanol exposures on subsequent voluntary ethanol intake in C57BL/6J mice. *Psychopharmacology (Berl)*, 181(4), 688–696. 10.1007/s00213-005-0026-3. [PubMed: 16001125]
- Lovinger DM, & Mathur BN (2012). Endocannabinoids in striatal plasticity. *Parkinsonism & Related Disorders*, 18(Suppl. 1), S132–S134. 10.1016/S1353-8020(11)70041-4. [PubMed: 22166411]
- Lu XY, Ghasemzadeh MB, & Kalivas PW (1998). Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience*, 82(3), 767–780. [PubMed: 9483534]
- Luscher C, & Malenka RC (2011). Drug-evoked synaptic plasticity in addiction: From molecular changes to circuit remodeling. *Neuron*, 69(4), 650–663. 10.1016/j.neuron.2011.01.017. [PubMed: 21338877]
- Maier EY, Kircher DM, Renteria R, Lasek AW, Wang YT, & Morrisett RA (2015). Lentivirus-induced accumbal overexpression of a GluA2 C-terminal peptide disrupts synaptic plasticity and alters operant and CIE-induced ETOH drinking. In 38th Annual scientific meeting of the research society on alcoholism, Abstract #198, 39(Issue S1), 61A.
- Maldve RE, Zhang TA, Ferrani-Kile K, Schreiber SS, Lippmann MJ, Snyder GL, et al. (2002). DARPP-32 and regulation of the ethanol sensitivity of NMDA receptors in the nucleus accumbens. *Nature Neuroscience*, 5(7), 641–648. 10.1038/nn877. [PubMed: 12068305]
- Malenka RC (2003). Synaptic plasticity and AMPA receptor trafficking. *Annals of the New York Academy of Sciences*, 1003, 1–11. [PubMed: 14684431]
- Malenka RC, & Nicoll RA (1999). Long-term potentiation—A decade of progress? *Science (New York)*, 285(5435), 1870–1874. 10.1126/science.285.5435.1870.
- Mangieri RA, & Morrisett RA (2015). Disruption of anaplastic lymphoma kinase activity attenuates LTD induction in medium spiny neurons (MSNs) in the nucleus accumbens shell. In 38th Annual scientific meeting of the research society on alcoholism, Abstract #216, 39(Issue S1), 65A <http://dx.doi.org/10.111/acer.12741>.
- Marinelli S, Pascucci T, Bernardi G, Puglisi-Allegra S, & Mercuri NB (2005). Activation of TRPV1 in the VTA excites dopaminergic neurons and increases chemical- and noxious-induced dopamine release in the nucleus accumbens. *Neuropsychopharmacology*, 30(5), 864–870. 10.1038/sj.npp.1300615. [PubMed: 15562294]
- Matamalas M, Bertran-Gonzalez J, Salomon L, Degos B, Deniau JM, Valjent E, et al. (2009). Striatal medium-sized spiny neurons: Identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. *PloS One*, 4(3), e4770 10.1371/journal.pone.0004770. [PubMed: 19274089]
- McCool BA (2011). Ethanol modulation of synaptic plasticity. *Neuropharmacology*, 61(7), 1097–1108. 10.1016/j.neuropharm.2010.12.028. [PubMed: 21195719]
- Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Look AT, et al. (1994). Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science (New York)*, 267(5151), 1281–1284.
- Morris SW, Naeve C, Mathew P, James PL, Kirstein MN, Cui X, et al. (1997). ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). *Oncogene*, 14, 2175–2188. [PubMed: 9174053]

- Morrisett RA, & Swartzwelder HS (1993). Attenuation of hippocampal long-term potentiation by ethanol: A patch-clamp analysis of glutamatergic and GABAergic mechanisms. *The Journal of Neuroscience*, 13(5), 2264–2272. [PubMed: 8478698]
- Mulkey RM, Herron CE, & Malenka RC (1993). An essential role for protein phosphatases in hippocampal long-term depression. *Science*, 261(5124), 1051–1055. [PubMed: 8394601]
- Musella A, De Chiara V, Rossi S, Prosperetti C, Bernardi G, Maccarrone M, et al. (2009). TRPV1 channels facilitate glutamate transmission in the striatum. *Molecular and Cellular Neurosciences*, 40(1), 89–97. 10.1016/j.mcn.2008.09.001. [PubMed: 18930149]
- Pascoli V, Turiault M, & Lüscher C (2012). Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature*, 481(7379), 71–75. 10.1038/nature10709.
- Pennartz CMA, Ameerun RF, Groenewegen HJ, & Lopes da Silva FH (1993). Synaptic plasticity in an in vitro slice preparation of the rat nucleus accumbens. *The European Journal of Neuroscience*, 5, 107–117. [PubMed: 7903183]
- Pulford K, Lamant L, Morris SW, Butler LH, Wood KM, Stroud D, et al. (1997). Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)-ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood*, 89(4), 1394–1404. [PubMed: 9028963]
- Renteria R, Jeanes ZM, & Morrisett RA (2014). Ethanol attenuation of long-term depression in the nucleus accumbens can be overcome by activation of TRPV1 receptors. *Alcoholism, Clinical and Experimental Research*, 38(11), 2763–2769. 10.1111/acer.12542.
- Renteria R, Maier EY, Buske TR, & Morrisett RA (2016). Selective alterations of NMDAR function and plasticity in D1 and D2 medium spiny neurons in the nucleus accumbens shell following chronic intermittent ethanol exposure. *Neuropharmacology* 10.1016/j.neuropharm.2016.03.004.
- Rhodes JS, Best K, Belknap JK, Finn DA, & Crabbe JC (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiology and Behavior*, 84(1), 53–63. 10.1016/j.physbeh.2004.10.007. [PubMed: 15642607]
- Robbe D, Kopf M, Remaury A, Bockaert J, & Manzoni OJ (2002). Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America*, 99(12), 8384–8388. 10.1073/pnas.122149199. [PubMed: 12060781]
- Robinson TE, & Berridge KC (2003). Addiction. *Annual Review of Psychology*, 54, 25–53. 10.1146/annurev.psych.54.101601.145237.
- Rodd-Henricks ZA, McKinzie DL, Li T-K, Murphy JM, & McBride WJ (2002). Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. *The Journal of Pharmacology and Experimental Therapeutics*, 303(3), 1216–1226. 10.1124/jpet.102.038950. [PubMed: 12438546]
- Rohrbough J, & Broadie K (2010). Anterograde Jelly belly ligand to Alk receptor signaling at developing synapses is regulated by Mind the gap. *Development (Cambridge, England)*, 137(20), 3523–3533. 10.1242/dev.047878.
- Ross RA (2003). Anandamide and vanilloid TRPV1 receptors. *British Journal of Pharmacology*, 140(5), 790–801. 10.1038/sj.bjp.0705467. [PubMed: 14517174]
- Savarese A, Zou ME, Kharazia V, Maiya R, & Lasek AW (2014). Increased behavioral responses to ethanol in Lmo3 knockout mice. *Genes, Brain, and Behavior*, 13(8), 777–783. 10.1111/gbb.12176.
- Scholz R, Berberich S, Rathgeber L, Kollek A, Köhr G, & Kornau HC (2010). AMPA receptor signaling through BRAG2 and Arf6 critical for long-term synaptic depression. *Neuron*, 66(5), 768–780. 10.1016/j.neuron.2010.05.003. [PubMed: 20547133]
- Schotanus SM, & Chergui K (2008). Long-term potentiation in the nucleus accumbens requires both NR2A- and NR2B-containing N-methyl-D-aspartate receptors. *European Journal of Neuroscience*, 27(8), 1957–1964. 10.1111/j.1460-9568.2008.06173.x. [PubMed: 18412616]
- Sellings LH, & Clarke PB (2003). Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. *The Journal of Neuroscience*, 23(15), 6295–6303. doi: 0270-6474/03/236295-09 [pii]. [PubMed: 12867514]
- Sesack SR, & Grace AA (2010). Cortico-basal ganglia reward network: Microcircuitry. *Neuropsychopharmacology*, 35(1), 27–47. 10.1038/npp.2009.93. [PubMed: 19675534]

- Smith RJ, Lobo MK, Spencer S, & Kalivas PW (2013). Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). *Current Opinion in Neurobiology*, 23(4), 546–552. 10.1016/j.conb.2013.01.026. [PubMed: 23428656]
- Spanagel R, & Weiss F (1999). The dopamine hypothesis of reward: Past and current status. *Trends in Neurosciences*, 22(11), 521–527. 10.1016/S0166-2236(99)01447-2. [PubMed: 10529820]
- Spiga S, Talani G, Mulas G, Licheri V, Fois GR, Muggironi G, et al. (2014). Hampered long-term depression and thin spine loss in the nucleus accumbens of ethanol-dependent rats. *Proceedings of the National Academy of Sciences of the United States of America*, 111(35), E3745–E3754. 10.1073/pnas.1406768111. [PubMed: 25122682]
- Thomas MJ, Beurrier C, Bonci A, & Malenka RC (2001). Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. *Nature Neuroscience*, 4(12), 1217–1223. 10.1038/nn757. [PubMed: 11694884]
- Thomas MJ, Malenka RC, & Bonci A (2000). Modulation of long-term depression by dopamine in the mesolimbic system. *The Journal of Neuroscience*, 20(15), 5581–5586. [PubMed: 10908594]
- Tsai LT-Y, Bainton RJ, Blau J, & Heberlein U (2004). Lmo mutants reveal a novel role for circadian pacemaker neurons in cocaine-induced behaviors. *PLoS Biology*, 2(12), e408 10.1371/journal.pbio.0020408. [PubMed: 15550987]
- Valjent E, Bertran-Gonzalez J, Herve D, Fisone G, & Girault JA (2009). Looking BAC at striatal signaling: Cell-specific analysis in new transgenic mice. *Trends in Neurosciences*, 32(10), 538–547. 10.1016/j.tins.2009.06.005.
- Wang K-S, Liu X, Zhang Q, Pan Y, Aragam N, & Zeng M (2011). A meta-analysis of two genome-wide association studies identifies 3 new loci for alcohol dependence. *Journal of Psychiatric Research*, 45(11), 1419–1425. [PubMed: 21703634]
- Weiss F, Lorang MT, Bloom FE, & Koob GF (1993). Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: Genetic and motivational determinants. *The Journal of Pharmacology and Experimental Therapeutics*, 267(1), 250–258. [PubMed: 8229752]
- Wise RA (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience*, 5(6), 483–494. 10.1038/nrn1406. [PubMed: 15152198]
- Wolf ME (2002). Addiction: Making the connection between behavioral changes and neuronal plasticity in specific pathways. *Molecular Interventions*, 2(3), 146–157. 10.1124/mi.2.3.146. [PubMed: 14993375]
- Yao S, Cheng M, Zhang Q, Wasik M, Kelsh R, & Winkler C (2013). Anaplastic lymphoma kinase is required for neurogenesis in the developing central nervous system of zebrafish. *PloS One*, 8(5), e63757 10.1371/journal.pone.0063757. [PubMed: 23667670]
- Zhang TA, Hendricson AW, & Morrisett RA (2005). Dual synaptic sites of D(1)-dopaminergic regulation of ethanol sensitivity of NMDA receptors in nucleus accumbens. *Synapse (New York)*, 58(1), 30–44. 10.1002/syn.20181.
- Zhou L, Furuta T, & Kaneko T (2003). Chemical organization of projection neurons in the rat accumbens nucleus and olfactory tubercle. *Neuroscience*, 120(3), 783–798. 10.1016/S0306-4522(03)00326-9. [PubMed: 12895518]
- Zschenderlein C, Gebhardt C, von Bohlen Und Halbach O, Kulisch C, & Albrecht D (2011). Capsaicin-induced changes in LTP in the lateral amygdala are mediated by TRPV1. *PloS One*, 6(1), e16116 10.1371/journal.pone.0016116. [PubMed: 21249195]

Fig. 1.

(A) Low-frequency conditioning stimulation paired with postsynaptic depolarization produces LTD of AMPAR-mediated EPSCs. This form of LTD relies on activation of NMDARs and influx of Ca^{2+} . Ca^{2+} influx recruits clathrin and adaptor proteins to bind to the intracellular tail of the GluA2 subunit C-terminal, resulting in internalization of GluA2-containing AMPARs. (B) The artificial peptide GluA2_{3Y} mimics this tail of the GluA2 subunit, saturates adaptor proteins, prevents AMPAR internalization, and therefore occludes formation of LTD. *This figure was derived from one generously provided by Dr. Yu Tian Wang.*

Fig. 2. CIE-induced metaplasticity of glutamatergic transmission differs between NAc shell D1 MSNs (*left panel*) and D2 MSNs (*right panel*). (A) In slices from ethanol-naïve mice, NMDAR-dependent LTD is observed in D1 MSNs following low-frequency stimulation (LFS) paired with postsynaptic depolarization to -50 mV. LTD is expressed via removal of AMPARs from the postsynaptic membrane. (B) Following in vivo CIE exposure the same LTD induction protocol that was applied to ethanol-naïve slices induces LTP of glutamatergic transmission onto D1 MSNs, presumably via insertion of AMPARs into the postsynaptic membrane. (C) LFS paired with postsynaptic depolarization does not elicit

LTD in D2 MSNs. (D) Following in vivo CIE exposure, however, this protocol does elicit LTD in D2 MSNs. It is not yet known the underlying mechanism mediating this form of LTD. For all panels, traces next to postsynaptic neurons represent an average amplitude-evoked EPSC during baseline or postpairing periods; corresponding scale bars indicate 5 ms (horizontal) and 50 pA (vertical). “?” signifies the unknown mechanism of LTD in D2 MSNs as stated in (D).

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Table 1

Accumbal Plasticity Screening

Background Strain	Manipulation		Postpairing EPSC Amplitude	
Line	Genetic	In Vitro	Ethanol Exposure	(% Baseline)
C57BL/6J				
			Naïve	65 ^a
			CIE	125 ^a
	CD14 KO		Naïve	45 ^a
	BKβ1 KO		Naïve	55 ^a
<i>Drd1a</i> -tdTomato			Naïve	50
			CIE	125
<i>Drd1a</i> -tdTomato		0 nM TAE684	Naïve	55
		5 nM TAE684	Naïve	75
		25 nM TAE684	Naïve	95
<i>Drd1a</i> -tdTomato × <i>Alk</i>	ALK WT		Naïve	45
	ALK KO		Naïve	75
Swiss Webster				
<i>Drd1a</i> -eGFP			Naïve	60
			CIE	95
HS/Npt^b				
HS/Npt control stock	No selection		Naïve	50
			CIE	50
HDID	Selective		Naïve	50
	Breeding of line		CIE	95

^aExperiments were performed on unidentified NAc shell MSNs. Ethanol-naïve, wild-type C57Bl/6J mice show NMDAR-LTD that corresponds to a postpairing EPSC amplitude of ~65% of baseline (first row). As described in Jeanes et al. (2014), this population of neurons can be divided into those that show “large LTD” or “small LTD” (average postpairing EPSC amplitudes of 55% or 90%), presumed to be D1 or D2 MSNs, respectively. For BKβ1 and CD14 KO experiments, unidentified MSNs were classified as having large or small LTD, and values reported in table represent the averages of large LTD MSNs.

^bExperiments were performed on D1 MSNs identified by a fluorescent retrograde tracer (cholera toxin subunit B conjugated to Alexa Fluor 555 injected into the VTA, see text).

EPSC amplitudes are the average of the 20- to 30-min postpairing period during which LTD is expressed and is reported as a percentage of the baseline average and rounded to the nearest 5%. Neurons were identified as D1 MSNs by epifluorescent illumination of a genetically encoded fluorescent reporter (ie, tdTomato or eGFP), with the following exceptions.