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Abstract

Early life experiences affect the formation of neuronal networks, which can have a profound impact on brain function and behavior later in life. Previous work has shown that mice exposed to excessive sensory stimulation during development are hyperactive, novelty-seeking and display impaired cognition compared to controls. In this study, we addressed the issue of whether excessive sensory stimulation during development could alter behaviors related to addiction and underlying circuitry in CD-1 mice. We found that the reinforcing properties of cocaine were significantly enhanced in mice exposed to excessive sensory stimulation. Moreover, although these mice displayed hyperactivity that became more pronounced over time, they showed impaired persistence of cocaine-induced locomotor sensitization. These behavioral effects were associated with alterations in glutamatergic transmission in the nucleus accumbens and amygdala. Together, these findings suggest that excessive sensory stimulation in early life significantly alters drug reward and the neural circuits that regulate addiction and attention-deficit hyperactivity. These observations highlight the consequences of early-life experiences and may have important implications for children growing up in today's complex technological environment.

Significance statement

Environmental stimulation in the form of enrichment has been shown to be beneficial for brain development and behavior. Although this has been broadly interpreted as stimulating the developing brain is positive, recent work demonstrates that sensory stimulation can in fact have negative consequences, particularly if it is non-normative, extensive and presented during development. This research adds to existing knowledge on the impact of early-life experiences and provides fundamental insights into how environmental factors during development can shape the brain and behavior. At a point where childhood and adolescence is increasingly dominated by exposure to audio-visual media, we believe our findings build the case for further investigation on the effects of extended exposure to sensory experiences in early life.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) and drug addiction are neuropsychiatric diseases with a high comorbidity rate and a strong genetic component (Capusan et al., 2016). However, there remains a large role for environmental factors in the etiology of these diseases (McCrory and Mayes, 2015). It is widely recognized that early life experiences shape neural function, which can have lasting impacts on behavior and vulnerability to developing these diseases. For example, childhood stress during periods of critical development increases propensity to impulsive choice, ADHD and drug use/abuse later in life, whereas positive life experiences such as good family and peer relations, can be protective against the development of ADHD and decrease the likelihood of drug use (Jessor and Jessor, 1980; Kodjo and Klein, 2002; Sinha, 2008; Enoch, 2012). Studies in rodent models have found similar effects. Animals exposed to stress early in life show impulsivity, impaired decision-making, greater motivation to seek drugs and increased rates of drug-induced reinstatement (McEwen, 2003; Ruedi-Bettschen et al., 2006; Andersen and Teicher, 2009). On the other hand, rodents reared in an enriched environment, which provides plenty of complex inanimate and social stimulation, have enhanced decision-making and cognition, decreased

83 motivation to seek drugs and lower rates of drug-induced reinstatement (Solinas et al., 2010; Takuma et al.,
84 2011).

85 Although much of the laboratory animal work on environmental risk factors has focused on
86 impoverished versus enriched environments, recent studies in humans have shown that exposure to
87 extensive periods of auditory and visual stimulation during childhood is highly correlated with attentional
88 problems (Christakis et al., 2004; Zimmerman and Christakis, 2007). However, human studies cannot be
89 used to establish a causal relationship between excessive sensory exposure and behavioral consequences. As
90 such, we have only a limited understanding of how increased sensory stimulation alters brain function,
91 behavior and changes risk to neuropsychiatric illness. While the introduction of animal models to study the
92 consequences of an enriched environment has led to deep and detailed insights into the underlying cellular
93 mechanisms, we know very little about the consequences of excessive sensory stimulation. Only two recent
94 studies have investigated the effects of repetitive sensory stimulation. One study showed that repetitive
95 olfactory stimulation during development in rats impaired performance in an attention task in the presence
96 of an auditory distractor (Hadas et al., 2016). Using repetitive auditory and visual stimulation in a mouse
97 model, the second study reported that extended exposure to sensory stimulation during development
98 produces pronounced hyperactivity, impaired cognition and increased novelty-seeking (Christakis et al.,
99 2012). In the present study, we have used the same mouse model to examine the effects of excessive
100 exposure to sensory stimulation during development on the rewarding and psychomotor activating effects of
101 cocaine, using conditioned place preference and locomotor sensitization, respectively. In addition, we
102 characterized whether this stimulation protocol produces baseline changes in neural activity in two
103 components of the neural circuits thought to contribute to addiction and ADHD, the nucleus accumbens
104 (NAc) and the amygdala.

105 **Materials and Methods**

106 **Experimental Animals**

107 Male CD-1 mice purchased from Charles River Laboratories (RRID:SCR_013551) were used for all
108 experiments. Mice (post-weaning) were group-housed (3-5 per cage) with *ad libitum* access to food and

water under a 12 h light/dark cycle (light on at 7:00 am) with controlled temperature (22 +/- 1 Celsius). All experiments and animal procedures were performed in accordance with the [Author University] animal care committee's regulations and conducted in accordance with the US National Institutes of Health guidelines.

Excessive Sensory Stimulation (ESS) Paradigm

Mice received sensory stimulation in their home cages for 42 consecutive days starting at P10. The stimulation occurred during the dark cycle for 6 hours per day. The dam was stimulated along with the pups from P10 until weaning (P21). Control groups were raised under standard laboratory housing conditions and tested at corresponding times with the sensory stimulation groups. The sensory stimulation set-up consisted of two loud speakers, suspended two inches above the top of the cage. Auditory stimulation consisted of audio from television cartoon shows (e.g., Pokemon, Powerpuff girls, Bakugan) which were layered on top of each other with one pitch shifted (10-20KHz), and one non pitch shifted track in order to better accommodate the higher frequency hearing range of mice. Sounds were no louder than 70dB, which is significantly lower than common auditory stress model. Light-emitting diode lights (LED) (red, green, yellow and blue) placed around the cages to provide visual stimulation. A photorhythmic modulator was used to change the frequency of the blinking LED lights in concordance with the sound output from the speakers.

Behavioral Tests

Conditioned Place Preference (CPP): The CPP test is a classical Pavlovian conditioning procedure used to study the reinforcing effects of unconditioned stimuli (e.g., drugs, food). CPP was performed in a three-chamber place-preference box (ENV-3013, Med Associates) using an unbiased, three-phase design (pre-conditioning, conditioning and post-conditioning). The CPP test was conducted on control and ESS mice from P52-P56 (Pre-conditioning test: P52, Conditioning: P53-P55, Post-conditioning test: P56). The apparatus consisted of two large compartments separated by a central neutral compartment. The two lateral compartments differed in floor texture and wall pattern - vertically striped walls and stainless steel grid rods for flooring on one side and horizontally striped walls and metal mesh flooring on the other; the small central compartment had a smooth floor. During the pre-conditioning phase, mice were placed in the central

compartment and allowed 15 min free access to all compartments of the CPP box. During the conditioning phase, mice received twice daily (morning and afternoon) conditioning sessions for 3 days. On each conditioning day, mice were confined to one compartment for 15 min immediately following saline (morning) or cocaine (15mg/kg, *ip*, obtained from the National Institute on Drug Abuse) (afternoon) administration. The choice of compartment for saline/cocaine pairing was randomized and counterbalanced across groups. During the post-conditioning phase, mice were given 15 min free access to the CPP apparatus on the day following the final conditioning session. Time spent in the compartments was tracked using Noldus EthoVision XT 8.0. A CPP score was calculated for each mouse as the difference between pre-conditioning and post-conditioning time spent in the drug-paired compartment. A change in preference for the drug-paired compartment serves as an index of the reinforcing effects of cocaine.

Activity Assessment and Psychomotor Sensitization: Activity levels in mice and the psychomotor activating effects of cocaine were measured using locomotor activity boxes (8.5 X 17.5 X 9 inch) from San Diego Instruments (SDI) that contained regular ground corncob bedding on the floor. The Photobeam Activity System software (SDI) was used to track total crossovers in a 4x8 photobeam configuration which provided a measure of locomotor activity. To induce psychomotor sensitization, mice received 10 treatment sessions over a 2-week period (induction phase, P52-P65). During each session, mice were habituated to the locomotor chambers for 45 min followed by an injection of cocaine (15 mg/kg, *ip*) or saline and locomotor activity was monitored for 60 min. After a 2-week withdrawal period, all mice received an escalating dose challenge of cocaine (challenge phase). During this phase, mice received a 45 min habituation period, followed by sequential injections of saline, 10 mg/kg, and 20 mg/kg cocaine spaced 60 min apart. Locomotor activity was monitored for the entire duration of the session and total crossovers within the 60 min sessions were plotted and used for statistical analysis.

In Vitro **Slice Electrophysiology**

Slice electrophysiology experiments were conducted on control and ESS mice at P52-P70. Their brains were quickly removed under deep anesthesia and 350 µm thick coronal slices containing the NAc shell or the lateral (LA) and basal amygdala (BA) were prepared. We chose to study these particular sub-regions as a

vast body of literature shows that these brain regions are interconnected, are required for cocaine related behaviors, and cellular changes in these sub-regions are thought to underlie the behavioral effects of cocaine administration (Thomas et al., 2001; Fuchs et al., 2002; Kourrich and Thomas, 2009; Stuber et al., 2011; Lee et al., 2013; Hsiang et al., 2014). Slices were transferred to a submerged chamber containing artificial cerebrospinal fluid (aCSF) (In mM - 124 NaCl, 2.7 KCl, 26 NaHCO3, 0.4 NaH2PO4, 10 Glucose, 4 Sodium Ascorbate, 1.3 MgCl2 and 2 CaCl2) equilibrated with 95%O2-5%CO2 at room temperature. Slices were incubated for at least 1 hour before being transferred to a superfused recording chamber. Excitatory pyramidal neurons in the BA or medium spiny neurons in the NAc shell were visually identified using a Zeiss Axioskop 2 FS microscope with IR-DIC. Patch electrodes (3-6 MΩ) were pulled from borosilicate glass pipettes on a P-97 Flaming-Brown Micropipette Puller (Sutter Instruments) and filled with the voltage-clamp pipette internal solution (for mEPSCs: (in mM) - 120 CsOH, 120 Gluconic acid, 20 CsCl, 10 HEPES, 4 MgATP, and 0.3 NaGTP, 10 Phosphocreatine; pH 7.3, 300 mOsm, for mIPSCs: (in mM) - 140 CsCl, 10 10 HEPES, 10 Phosphocreatine , 4 MgATP, and 0.3 NaGTP; pH 7.3, 290 mOsm.). Whole-cell patch clamp recordings were performed using an Axon Multiclamp 700B patch-clamp amplifier. All recordings were performed at 30^0C. Neurons were voltage clamped at -70 mV. Miniature excitatory postsynaptic currents (mEPSCs) were isolated by using 75µM Picrotoxin and 0.5µM TTX in the aCSF solution, and miniature inhibitory postsynaptic currents (mIPSCs) were isolated by adding 10µM CNQX, 30µM D-APV and 0.5µM TTX in the aCSF solution. Continuous current traces were recorded for a 5 min period. Series resistance (Rs) was monitored before and after the experiment, and only cells with Rs value less than 25 MΩ were taken for analysis. Data was filtered at 2.1 kHz and digitized at 10 kHz. The amplitude and frequency of mEPSCs and mIPSCs were analyzed using the Mini Analysis Program (Synaptosoft). Firing output of BA neurons was measured in the current-clamp mode (Internal solution composition in mM – 140 K-gluconate, 10 HEPES, 1 $CaCl_2$, 2 $MgSO_4$, 4 Na_2ATP, 0.3 Na_2GTP 10 EGTA) and the membrane potential was adjusted to −70 mV before the injection of each current pulse. Action potential firing in response to a series of depolarizing current steps was recorded. Saturating current intensities were excluded from the analysis. Some basic properties of BA principal neurons were also measured in the current clamp mode. Resting membrane potential (Resting V_m) was measured immediately after achieving whole-cell configuration by

bringing the holding current to 0pA. Action potential threshold was estimated by injecting a ramp of current (0-500 pA in 100 ms) and measuring the voltage at which the first action potential occurred. Current-Voltage relationship (IV curve) was analyzed by measuring the peak voltage response to a series of current steps ranging from -100 to 50 pA. The input resistance was calculated as the slope of the IV curve for each neuron.

Corticosterone measurement

Plasma corticosterone (CORT) levels in control and mice that received sensory stimulation were quantified using an ELISA assay. Following 42 days of the sensory stimulation protocol, at age P52, mice were sacrificed and blood samples were collected for CORT measurements. Mice were anaesthetised with isoflorane and decapitated to collect trunk blood into lithium heparinized tubes (BD microcontainer 365971). The blood samples were then centrifuged at 10,500 rpm for 10 min at 4^{o}C to isolate plasma. The supernatant was then collected into Eppendorf tubes and stored at -80^{o}C until further analysis. To quantify CORT levels, the plasma sample were thawed and ELISA assays were performed by following the manufacturer's instructions (# KO14-H5, Arbor assays, RRID:SCR_013534).

Statistical analyses

Statistical analyses were conducted using either 2 WAY RM ANOVA with Bonferroni's *post hoc* analysis (to correct for multiple comparisons) or a one-sample or two-sample *t* test (without correction) when appropriate and as indicated, using GraphPad Prism (GraphPad, RRID:SCR_000306). Differences were considered statistically significant at $p \leq 0.05$.

Results

Exposure to excessive sensory stimulation during development enhances CPP to cocaine.

The rewarding effects of cocaine were assessed in controls and mice that received ESS using a CPP procedure; testing was performed in a drug-free state (**Fig. 1a**). We found that both groups of mice acquired a clear preference for the cocaine-paired chamber (**Fig. 1b;** CON: $t_{13} = 3.81$, $P = 0.002$; ESS: $t_{12} = 6.08$, $P < 0.0001$). However, mice that received sensory stimulation during development had a significantly greater

CPP score compared to controls (**Fig. 1b;** $t_{25} = 2.09$, $P = 0.04$), suggesting that they had a more robust response to the rewarding properties of cocaine.

Exposure to excessive sensory stimulation during development impairs the persistence of cocaine-induced locomotor sensitization.

In a separate cohort of mice, we assessed locomotor activity and the development of cocaine sensitization in control and ESS mice. As expected from other behavioral tests (Christakis et al., 2012), mice that received sensory stimulation during development were significantly more active than controls on the first day of saline treatment and this effect was stronger by the last test session (**Fig. 1c;** main effect of Stimulation: $F_{1,14} = 18.02$, $P = 0.0008$; $P < 0.05$ (session 1) and $P < 0.001$ (session 10) versus control). Thus, sensory stimulation during development led to hyperactivity that became increasingly more pronounced with repeated exposure to the testing environment. Given the differences in locomotor activity in saline groups, the responses of the cocaine groups were normalized to these different baselines (by subtracting the average total crossovers in the corresponding saline group from the total crossovers for each mouse) in order to gain a clearer picture of the impact of developmental sensory stimulation exposure on locomotor sensitization to cocaine. During the induction phase of sensitization, we found that the acute locomotor response to cocaine was decreased in mice that received extended periods of sensory stimulation during development compared to controls, although this effect did not quite reach statistical significance (**Fig. 1d, left;** main effect of Stimulation: $F_{1,19} = 10.82$, P = 0.004; $P = 0.1$ (session 1) versus control). Nonetheless, both groups showed significant increases in locomotor responses following repeated cocaine treatment, suggesting that sensitization had developed in all mice (**Fig. 1d, left;** main effect of Session: $F_{1,19} = 14.97$, $P = 0.001$; no interaction: $F_{1,19} = 0.71$, $P = 0.41$).

Following a 2-week withdrawal period, all mice underwent a challenge session, which included an injection of saline to test for the development of a conditioned response in mice that had previously received cocaine injections. As expected, control mice showed a conditioned locomotor response to this saline injection; however, mice that were exposed to the sensory stimulation protocol did not (**Fig. 1d, left**; main effect of Pretreatment: $F_{1,33} = 10.17$, $P = 0.003$; $P = 0.009$ versus saline-pretreated control). In addition, both groups of mice that received cocaine treatment during the induction phase showed greater locomotor

responses to the challenge doses of cocaine compared to the saline-treated mice. However, the cocaine-treated mice that received the sensory stimulation exposure during development had significantly decreased locomotor responses during the cocaine challenge compared to controls (**Fig. 1d, right**; 10 mg/kg: main effect of Pretreatment: $F_{1,33} = 36.47$, $P < 0.0001$; main effect of Stimulation: $F_{1,33} = 5.42$, $P = 0.03$, $P = 0.007$ versus cocaine-pretreated controls; 20 mg/kg: main effect of Pretreatment: $F_{1,33} = 27.57$, $P < 0.0001$, P = 0.05 versus cocaine-pretreated controls), suggesting that the persistence of sensitization was impaired. Thus, despite the fact that exposure to excessive sensory stimulation during development produces hyperactivity, it also results in blunted locomotor sensitization to cocaine.

Exposure to excessive sensory stimulation is not stressful.

Stress is a well-known modulator of the behavioral effects of cocaine (Shaham et al., 2000; Kreibich et al., 2009). Thus, to assess whether the stimulation paradigm results in a stress phenotype, body weights and plasma corticosterone (CORT) levels were measured in mice at P53 (i.e., 24 h following the last stimulation exposure). We found that body weights were the same in mice that were exposed to excessive periods of sensory stimulation during development and controls (**Fig. 2a;** $t_{69} = 1.30$, $P = 0.20$). In addition, there were no differences in plasma CORT levels between control mice and those that underwent the sensory stimulation protocol (**Fig. 2b;** $t_{18} = 0.93$, $P = 0.37$). These observations suggest that the extended exposure to lights and sounds used in the sensory stimulation protocol does not alter baseline stress levels in the mice.

Exposure to excessive sensory stimulation during development increases the frequency of miniature EPSCs in limbic circuits.

To begin to explore neural correlates of the observed behavioral changes, we next examined whether exposure to excessive sensory stimulation produces a fundamental shift in neuronal activity by measuring miniature excitatory postsynaptic currents (mEPSCs) in the shell region of the NAc, as well as the lateral (LA) and basal (BA) nuclei of the amygdala. In the NAc (**Fig. 3)**, we found that while mEPSC amplitude was not different between groups (**Fig. 3e**, $t_{18} = 0.57$, $P = 0.58$), there was a significant increase in the frequency of mEPSC in the mice that received sensory stimulation during development compared to controls (**Fig. 3d**, $t_{18} = 4.67$, $P = 0.0002$).

Similarly, we found a significant increase in the frequency (t_{18} = 2.35, P = 0.03), but not the amplitude (t_{18} = 0.58, P = 0.57) of mEPSCs in the BA of young mice that had received sensory stimulation compared to controls (**Fig. 4a**). In contrast, we found no difference in the frequency (t_{18} = 0.68, P = 0.51) or amplitude (t_{18} = 1.45, P = 0.16) of mEPSCs in the LA (**Fig. 4b**). This observation was specific to excitatory currents in the BA as we observed no difference in either the frequency (t_{18} = 0.33, P = 0.74) or the amplitude (t_{18} = 0.89, P = 0.39) of miniature inhibitory postsynaptic currents (mIPSCs) in BA principal neurons (**Fig. 4c**). Interestingly, we found that the increase in mEPSC frequency ($t_{17.05}$ = 2.05, P = 0.05) in BA neurons persisted even 2 months after the end of stimulation, suggesting that these cellular changes are long-lasting (**Fig. 4d**).

In order to test the functional consequence of enhanced mEPSC frequency on BA neurons, we measured the firing output of BA principal neurons. Neurons were current-clamped with the membrane potential maintained at −70 mV and action potential firing in response to somatic injections of increasing steps of depolarizing currents was recorded (**Fig. 5a)**. We found that while there was a significant increase in firing rates with current injection across both groups, there was no significant difference in firing rates between cells from slices of mice that received excessive sensory stimulation during development and control mice (**Fig. 5b;** main effect of Current: $F_{10,110}$ = 24.38, P < 0.0001; no main effect of Stimulation: $F_{1,11}$ = 0.54, P = 0.48). Other basic properties measured in the current clamp mode, namely, resting membrane potential, action potential threshold, the I-V curve, and input resistance were not different between BA neurons in control and ESS mice (**Fig. 5c-f**). These findings indicate that excessive periods of sensory stimulation leads to a specific increase in the frequency of mEPSCs in the BA and the NAc.

Discussion

Early-life experiences have critical influences on the development of neural circuits and on susceptibility to drug use and addiction (Andersen and Teicher, 2009; Solinas *et al*, 2010). Understanding these influences is very important as early-life experiences not only drive adaptation, but under certain conditions, can be a major source of maladaptation. Enriched environments in rodents are known to be pro-cognitive, decrease addiction vulnerability and enhance brain function, whereas impoverished environments have the opposite

effects (Fabel and Kempermann, 2008; Kempermann et al., 2010; Volkers and Scherder, 2011). However, unlike the positive effects of an enriched environment, it has recently been shown that exposure to extended periods of sensory stimulation during development in mice produces ADHD-like symptoms including hyperactivity, impaired cognition, increased novelty-seeking and increased distractability (Christakis et al., 2012; Hadas et al., 2016). Here we found that exposure to excessive sensory stimulation also enhances the rewarding effects of cocaine while blunting its psychomotor activating effects. This is a significant finding, given the high comorbidity of ADHD and addiction (Zernicke et al., 2010; Jupp and Dalley, 2014). In addition, this result is consistent with work examining psychostimulant-induced locomotor activity and sensitization using other models of ADHD that express a hyperactive phenotype, such as the dopamine transporter (DAT) knockout mouse and the spontaneously hypertensive rat (SHR) (Sagvolden et al., 2005). However, it is possible that the enhanced CPP observed in the stimulated mice was due to alterations in learning and memory and this possibility will be explored in future studies.

In addition to these behavioral alterations, this excessive stimulation paradigm leads to a lasting enhancement in the frequency of mEPSCs in principal neurons of the amygdala and NAc - regions that are critical components of the neuronal circuits that regulate cognition, impulsivity and reward. Although profound and widespread, the neurobiological changes caused by excessive sensory stimulation are very specific. In particular, the baseline increases in mEPSC frequency in the BA and the NAc shell raises the intriguing possibility that excessive sensory experiences during childhood and adolescence lead to a fundamental shift in excitatory drive from sensory inputs to these regions, which in turn could affect the threshold for generating behavioral responses through downstream projections of these regions. Thus, because of an altered set-point, children exposed to excessive sensory stimulation may need higher levels of stimulation to elicit a behavioral action, which is reminiscent of children with ADHD. Dissecting the mechanisms underlying these changes, as well as how these alterations in baseline plasticity contributes to the dysregulated behaviors observed in sensory stimulation-induced attentional problems, ADHD and addiction, warrants future investigation.

The amygdala is an essential component of the circuitry that assigns emotional valence to external stimuli and produces appropriate behavioral responses (Aggleton, 2000; Phelps and LeDoux, 2005). It is

also an important part of the brain circuits that regulate learning and memory, anxiety and addiction (Davis, 1992; Roozendaal et al., 2009; Koob and Volkow, 2010), and aberrant amygdala activity is associated with numerous psychiatric illnesses, including ADHD and addiction (Kilts, 2001; Anand and Shekhar, 2003; King et al., 2003; See et al., 2003). In particular, the BA sub-region of the amygdala has been found to play a key role in behaviors related to drug addiction (Baxter and Murray, 2002; Fuchs et al., 2002; Tye and Deisseroth, 2012; Heldt et al., 2014; Hsiang et al., 2014). Similarly, the NAc is also a critical component of these circuits and changes in NAc activity are also associated with ADHD and addiction (Genro et al., 2010; Koob and Volkow, 2010). Specifically, the integration of dopaminergic reinforcement signals with glutamatergic signals (from the amygdala, hippocampus, medial prefrontal cortex and thalamus) that encode information about environmental stimuli leads to plasticity in the NAc that is thought to underlie motivation, reward and drug-taking and -seeking behaviors (Yager et al., 2015). Further, the shell region of the NAc is particularly important for mediating both the rewarding and psychomotor activating effects of cocaine (Pontieri et al., 1994; Caine et al., 1995; Pierce and Kalivas, 1995; Pontieri et al., 1995; McKinzie et al., 1999; Parkinson et al., 1999). Given that the BA and NAc are interconnected and can influence circuit function and plasticity, it is likely that the electrophysiological changes that occur in these brain regions following excessive sensory stimulation are contributing to the altered behavioral responses to cocaine (Stuber et al., 2011; Britt et al., 2012; MacAskill et al., 2014).

The sensory stimulation paradigm used in the present set of experiments does not appear to be inherently stressful to mice. The audio stimulation in this model (70 db) is well below the levels typically used in acoustic stress models (100–115 db). Moreover, stress leads to an increase in anxiety-like behavior (Conrad et al., 1999; Vyas and Chattarji, 2004), while a previous report has found that young mice receiving excessive periods of sensory stimulation show a decrease in anxiety-like behavior (Christakis et al., 2012). Stress can also affect body weight gain (Vyas et al., 2002; Gao et al., 2011); however, we found no difference in body weights between controls and mice exposed to sensory stimulation (**Fig. 3a**). In addition, repeated exposure to a stressor normally triggers a hypothalamic-pituitary-adrenal (HPA) axis response, leading to alterations in baseline plasma CORT levels (Odio and Brodish, 1989); yet we found that baseline plasma CORT levels in mice that received the sensory stimulation protocol were comparable to controls

(**Fig. 3b**). Thus, there is no indication that the neurobiological and behavioral effects reported here are caused by stress, experienced directly, or indirectly via maternal stress.

Understanding the impact of excessive exposure to sensory stimulation is highly relevant to today's society. Although animal models do not utilize the type of stimuli that rodents typically encounter under natural circumstances and cannot fully mimic the human experience, they have nonetheless contributed to a deep mechanistic understanding of the effects of environmental enrichment. Yet we have only very limited mechanistic insights into the consequences of exposure to sensory hyper-stimulation. Here we show that in the developing brain, excessive exposure to auditory and visual stimulation alters behavioral susceptibility to cocaine and changes baseline neuronal activity in associated neural circuits. It is conceivable that abnormally patterned stimulation or even too much sensory stimulation may contribute to the rise in ADHD diagnoses that are occurring in the past decade, which could in turn influence addiction rates. Interestingly, our research findings along with previous studies on sensory stimulation in rodents are reminiscent of clinical observations in children exposed to extensive television viewing and resemble the three core clinical dimensions of ADHD (inattentiveness, impulsivity and hyperactivity). In addition, stimulants such as Ritalin normalize the hyperactivity associated with ADHD and consistent with this we found that cocaine-induced locomotor sensitization was blunted in mice that received extended periods of sensory stimulation. Finally, children with ADHD have an increased risk for developing drug abuse and addiction (Harstad and Levy, 2014), and we found that mice that received sensory stimulation displayed an increase in the rewarding effects of cocaine, indicating an enhanced vulnerability to drugs of abuse. Thus, the excessive sensory stimulation paradigm provides a highly relevant model to understand how environments that contain excessive and ill-patterned stimuli influence behavioral outcomes, change neuroplasticity, and influence the propensity to develop neuropsychiatric disorders, such as ADHD and addiction.

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552 **Figure legends**

553 **Figure 1** Exposure to excessive sensory stimulation (ESS) during development alters behavioral responses
554 to cocaine and locomotor activity. **(a)** Representative heat map of time spent in the different compartments
555 of the conditioned place preference (CPP) box during the pretest (left) and on the test (right). **(b)** Mice
556 exposed to ESS during development had a significantly greater CPP score compared to controls (CON) (**P*
557 < 0.05 versus CON, n=13-14/group). **(c)** Locomotor activity following saline administration in CON (white
558 circles) and ESS (white squares) mice, as measured by the total number of crossovers**.** Exposure to ESS
559 during development led to a significant increase in locomotion compared to controls (**P* < 0.05 versus CON
560 session 1; ****P* < 0.001 versus CON session 10, n=7-9/group). **(d**) *Left, Induction phase:* Total number of
561 crossovers made during the 60 min following cocaine injection normalized to baseline responding (i.e.,
562 average total crossovers in the corresponding saline group was subtracted from total crossovers for each
563 mouse) in control (black circles) and ESS (black squares) mice. Exposure to ESS during development
564 significantly attenuated the development of locomotor sensitization during cocaine treatment (***P* < 0.01
565 versus CON session 10, n=10-11 mice/group). *Right, Challenge phase:* Total number of crossovers made
566 during the 60 min following each dose of a multi-dose challenge (0, 10 and 20 mg/kg cocaine). Responses
567 normalized to corresponding saline pretreatment group at the 0 mg/kg challenge. Control mice that received
568 cocaine during the induction phase, but not mice that were exposed to ESS during development, displayed a
569 conditioned locomotor response (*##P* = 0.009 versus saline-pretreated CON*)*. In addition, ESS mice showed
570 a significantly blunted locomotor sensitization to cocaine (***P* = 0.007 versus cocaine-pretreated CON; **P*
571 = 0.05 versus cocaine-pretreated CON session 10, n=7-11 mice/group). Data represent mean +/- SEM.

572 **Figure 2** Exposure to excessive sensory stimulation (ESS) does not effect measures of a stress response. (**a**)
573 Exposure to ESS during development does not alter body weight at P53 (i.e. the day after the end of ESS
574 exposure) compared to controls (CON) (n=32-39 mice/group). **(b)** Plasma CORT levels at P53. Exposure to
575 ESS during development does not affect baseline plasma CORT levels compared to CON (n=10
576 mice/group). Data represent mean +/- SEM.

577 **Figure 3** Excessive sensory stimulation (ESS) enhances excitatory tone in the nucleus accumbens (NAc)
578 shell. **(a)** Representative miniature excitatory postsynaptic current (mEPSC) traces from NAc shell neurons
579 in slices from CON and ESS mice. **(b,c)** Cumulative probability distribution for inter-event interval (b) and
580 amplitude (c) of mEPSCs in NAc shell neurons. **(d,e)** Exposure to ESS during development significantly
581 increased the frequency (***P = 0.0002, n=9-11 cells/group, N= 3-4 mice/group) but not the amplitude of
582 mEPSCs in the NAc shell compared to CON. Scale bar = 20pA (vertical axis), 50ms (horizontal axis). Data
583 represent mean +/- SEM.

584 **Figure 4** Excessive sensory stimulation (ESS) during development enhances excitatory tone in the basal
585 amygdala. **(a, b)** *Top:* Representative miniature excitatory postsynaptic current (mEPSC) traces from BA (**a**)
586 and LA (**b**) principal neurons in slices from ESS and CON mice. *Bottom:* Exposure to ESS during
587 development significantly increased the frequency of mEPSCs in the BA *(***a***, left,* *P = 0.03, n=16-17
588 cells/group, N=4-9 mice/group) but not in the LA (**b***, left,* n=10 cells/group, N=3-4 mice/group) compared to
589 CON. There was no effect of this manipulation during development on the amplitude of mEPSCs in the BA
590 *(***a***, right*) or in the LA (**b***, right*). *Center:* Cumulative probability distribution for inter-event interval (left)
591 and amplitude (right) of mEPSCs in BA (**a**, center) and LA (**b**, center) neurons. **(c)** *Top:* Representative
592 miniature inhibitory postsynaptic current (mIPSC) traces from BA principal neurons in slices from ESS and
593 CON mice. *Bottom:* Exposure to ESS during development had no effect on the frequency *(left)* or the
594 amplitude *(right)* of mIPSCs in the BA compared to CON (n=8-10 cells/group, N=3-4 mice/group). *Center:*
595 Cumulative probability distribution for inter-event interval (left) and amplitude (right) of mIPSCs in BA
596 neurons. **(d)** *Top:* Representative mEPSC traces from BA principal neurons in slices from adult ESS and
597 CON mice 2 months after the end of the stimulation protocol. *Bottom:* The mEPSC frequency (**d**, *left,* *P =
598 0.05, n=11-13 cells/group, N=3-4 mice/group) but not amplitude *(right)* was significantly increased 2
599 months following the end of ESS. *Center:* Cumulative probability distribution for inter-event interval (left)
600 and amplitude (right) of mEPSCs in BA neurons 2 months following the end of ESS. Scale bar (b,c,d,e) =
601 20pA (vertical axis), 50ms (horizontal axis). Data represent mean +/- SEM.

Figure 5 Exposure to excessive sensory stimulation (ESS) does not change action potential firing or basic properties of basal amygdala (BA) principal neurons. **(a)** Representative spike trains evoked by somatic injection of increasing steps of depolarizing currents. **(b)** Input-Output (I-O) curve (number of action potentials versus current injected) for BA principal neurons in slices from mice exposed to ESS during development (black squares) and CON (white circles). There were no differences in the I-O curve between groups (n=6-7 cells/group, N=3-4 mice/group). **(c-f)** Basic properties of BA principal neurons recorded from control and ESS brain slices. **(c)** Resting membrane potential (Resting Vm) was not different between BA principal neurons in ESS and CON brain slices (n=6-7 cells/group, N=3-4 mice/group). **(d)** Action potential threshold (mV) was not different between BA principal neurons in ESS and CON brain slices (n=6 cells/group, N=3-4 mice/group). **(e)** The IV curve (current-voltage relationship) was not different between BA principal neurons in ESS and CON brain slices (n=5-6 cells/group, N=3 mice/group). **(f)** Input resistance was not different between BA principal neurons in ESS and CON brain slices (n=5-6 cells/group, N=3 mice/group). Scale bar (a) = 40mV (vertical axis), 100ms (horizontal axis). Error bars indicate mean +/- SEM.