Neuroimaging reveals functionally distinct neuronal networks associated with high level alcohol consumption in two genetic rat models

Short title: Strain-specific functional brain activation by alcohol in rats

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ABSTRACT

Human imaging data suggest that the motivational processes associated with alcohol reward are reflected in the patterns of neural activation after alcohol or alcohol-related cues. In animal models of alcohol drinking, however, the changes in brain activation during voluntary alcohol ingestion are poorly known. In order to improve the translational utility of animal models, we examined alcohol-induced functional brain activation in AA (Alko Alcohol) and Marchigian-Sardinian alcohol-preferring (msP) rats that drink voluntarily high levels of alcohol, but exhibit widely different neurochemical and behavioral traits co-segregated with alcohol preference. Brain imaging was performed using manganese-enhanced magnetic resonance imaging (MEMRI), which is based on accumulation of Mn^{2+} ions in activated neurons, allowing the identification of functional neuronal networks recruited during specific behaviors in awake animals during a subsequent imaging session under anesthesia. MEMRI was performed following four weeks of voluntary alcohol drinking, using water drinking as the control. Despite similar levels of alcohol drinking, strikingly different alcohol-induced neuronal activity patterns were observed in AA and msP rats. Overall, functional activation in the AA rats was more widespread, involving large cortical areas and subcortical structures, such as the bed nucleus of the stria terminalis, preoptic area, hypothalamus, periaqueductal grey, and substantia nigra. In the msP rats, however, alcohol-related activation was largely confined to prefrontal cortical regions and insular cortex, and olfactory areas. Overlapping areas of activation found in both rat lines included the nucleus accumbens, prelimbic, orbital, and insular cortex. In conclusion, our data reveal strikingly different brain circuits associated with alcohol drinking in two genetically different rat lines and suggest innately different motivational and behavioral processes driving alcohol drinking. These findings have important implications for the use of these lines in translational alcohol research.

KEYWORDS: alcohol drinking; functional brain activity; selected rat lines; manganese-enhanced magnetic resonance imaging

INTRODUCTION

Functional brain imaging in humans reveals that patients suffering from alcohol use disorders (AUD) display differences from healthy controls in brain systems associated with reward and motivation, as well executive and inhibitory control. In AUD patients, alcohol or alcohol-associated cues activate ventral or dorsal striatum, regions of the prefrontal cortex, insula, and thalamus (Myrick et al., 2004; Filbey et al., 2008; Gilman et al., 2008; Vollstadt-Klein et al., 2010; Gilman et al., 2012a; Gilman et al., 2012b; Courtney et al., 2016; Bach et al., 2020). Functional neuroimaging has also begun to identify biomarkers for AUD pharmacotherapies by evaluating brain activation changes induced by pharmacological treatments (Grodin and Ray, 2019). These studies have both highlighted the mechanisms of action of the pharmacological agents used for treatment and predicted clinical response (Schacht et al., 2017; Bach et al., 2020).

The findings in AUD are compatible with the neurocircuitry of alcohol reinforcement and dependence established with various methods in rodents (Spanagel, 2009). Rodents will voluntarily drink alcohol to attain pharmacologically and behaviorally relevant blood alcohol concentrations, and the ensuing activation of brain circuits can be measured. However, brain activation measured noninvasively in freely moving subjects would greatly enhance the translational use of data from animal models. Neuroimaging can objectively measure activity in brain circuits involved in alcohol drinking and both elucidate the neural circuitry underlying the incentives to drink alcohol and offer biomarkers of the actions of novel pharmacological agents for further human testing. Thus, biomarkers from *in vivo* neuroimaging methods can provide objective measures of distinct animal phenotypes that allow more straightforward translation into humans than behavioral responses, and may thus provide highly needed translational biomarkers (Heilig et al., 2016).

The use of *in vivo* neuroimaging techniques in rodents for mapping brain activity, including Magnetic Resonance Imaging (MRI), has been limited by the necessity of anesthesia for animal fixation in imaging settings. However, the emerging manganese-enhanced MRI (MEMRI) protocols offer a feasible approach (Silva et al., 2004). Paramagnetic Mn^{2+} ions enter excitable cells during depolarization through voltage-gated calcium channels. The activity-dependent accumulation of

Mn²⁺ in neurons reports activated brain areas as increased signal intensity in T₁-weighted MR images. Because Mn²⁺ does not leave the areas for several hours, the pattern of activation is retained. Therefore, neuronal activation resulting from behavioral activity in freely moving animals can be subsequently measured under anesthesia using MEMRI (Eschenko et al., 2010b; Dudek et al., 2015; Dudek et al., 2016).

Selective breeding was an early approach for developing rodent models consuming high amounts of alcohol (Mardones et al., 1953; Eriksson, 1968). Many breeding programs produced rat lines that quickly learn to drink voluntarily high amounts of alcohol (5-8 g/kg/day) that can lead to blood alcohol levels as high as 100 mg% (0.1g/100 ml) (Ciccocioppo, 2013). Despite their similar alcohol preference phenotypes, rats from different selection programs differ in distinct neurochemical and behavioral traits which have co-segregated with their alcohol preference. For example, the Finnish alcohol-preferring AA rats are impulsive and risk-taking (Moller et al., 1997; Roman et al., 2007), whereas the Sardinian preferring line and its substrain, the Marchigian-Sardinian alcohol-preferring rats (msP), exhibit an anxiogenic- and depression-like phenotype (Colombo et al., 1995; Hansson et al., 2006). These rats display a constitutive overexpression of brain CRF1 receptors that is normalized following voluntary alcohol drinking (Hansson et al., 2006; Hansson et al., 2007).

Here, we hypothesized that MEMRI could capture neuronal activity patterns resulting from voluntary alcohol drinking by the alcohol-preferring rat lines. We then aimed at comparing functional brain activation in the AA and msP rats in response to alcohol consumption. Given their widely different behavioral traits besides similar alcohol-drinking phenotypes, differences in brain activation may help to decipher disparate motivational mechanisms involved in their alcohol consumption. Because alcoholism is an etiologically and clinically heterogeneous disorder (Heilig et al., 2011), comparison of the genetically different high-alcohol drinking phenotypes could highlight neurobiological mechanisms underlying this diversity and present brain activation biomarkers for testing the therapeutic potential of novel medications.

METHODS

Animals

Twenty male alcohol-preferring AA (Alko, Alcohol) rats (265-335 g at the beginning of the experiments) and 28 msP rats (370-480 g) were used. Experiments with AA rats were performed at the Department of Pharmacology of the University of Helsinki, and msP rat experiments were performed at the Neuroscience Institute of the Superior Research Council of Spain and Miguel Hernández University. In all cases, rats were individually housed in transparent polycarbonate cages with bedding material, and a wooden stick and nesting material were given as enrichment. Rats had ad libitum access to food and water and were housed under controlled temperature ($21 \pm 1^\circ\text{C}$) and relative humidity ($55 \pm 10\%$) on a 12-hour light/dark cycle. All experimental procedures using animals were approved by local authorities (State Provincial Office of Southern Finland and IN-CSIC ethical committee, respectively) and carried out in accordance with the European Union Commission recommendation (2007/526/EC), the Finnish Act on the Use of Animals for Experimental Purposes and the Spanish law 32/2007.

Voluntary alcohol consumption

Drinking fluids were provided in 250-ml drinking bottles equipped with stainless steel spouts, placed in the food tray of the cage covers. During the first 4 days, AA rats of the alcohol group were given 10% (v/v) ethyl alcohol as their only drinking fluid to habituate them to the taste of alcohol. This habituation is a standard procedure in the AA rats, but has not deemed necessary for msP rats. All rats were allowed a 2-bottle choice between 10% (v/v) alcohol and water for the next 4 weeks, during which alcohol consumption reached a plateau. Twice per week, consumption of the fluids and body weights were recorded, and the bottles were filled with fresh solutions. During this phase,

the water-drinking control group had always access to water in two drinking bottles. During the MnCl_2 infusion, liquid consumption and body weights were recorded daily.

MnCl_2 administration

Different doses and administration protocols for MnCl_2 (Eschenko et al., 2010b; Eschenko et al., 2010a) were first tested in both rat strains in order to optimize MEMRI experiments in combination with alcohol drinking paradigms. The optimization process traded off between two factors: achievement of minimal or no MnCl_2 side effects, measured in general behavioral parameters with special attention to the achieved drinking levels, and maximization of T_1 -weighted signal enhancement in MR images. In all cases MnCl_2 was administered as an isotonic solution dissolved in Tris-buffered saline (pH 7.4). As a consequence of this optimization process, we chose osmotic minipump administration (ALZET® model 2001) delivering 200 μl of MnCl_2 (1 $\mu\text{l}/\text{h}$) during a 7-day infusion period. The total dose of infused MnCl_2 was 120 mg/kg for AA and 80 mg/kg for msP rats, increasing the signal-to-noise ratios (SNR) in imaging data by a factor of 1.25 and 1.40, respectively, with no toxic effects (see Results section). Before the surgery, the pumps were primed overnight in a 37°C saline solution. The pumps were implanted subcutaneously on the dorsum, slightly caudal to the scapulae. The animals received post-surgical analgesia (Carprofen, 5 mg/kg) immediately after implantation and were returned to their home cages with access to water only (control groups) or both alcohol and water bottles (alcohol-exposed groups). All animals were imaged immediately following the 7-day MnCl_2 infusion within 24 h of termination of alcohol access.

MRI data acquisition

Anesthesia was induced with 4-5% isoflurane in oxygen (0.8-1 L/min), and animals were secured on custom-made holding apparatus with a tooth bar and a nose cone. During scanning, the isoflurane

concentration was maintained at 2%, and the body temperature was kept constant with a heating pad.

MRI experiments on AA rats were performed on a 4.7 T scanner (Bruker, PharmaScan 47/16, Ettlingen, Germany) using a 38 mm linear volume coil for transmit and receive. T_1 -weighted images were acquired using a rapid acquisition-relaxation enhanced (RARE) pulse sequence (TR = 300 ms, TE = 12.5 ms, averages = 7, field of view FOV = 26 x 17 x 17 mm, matrix size = 128 x 54 x 54, resulting in 0.2 x 0.31 x 0.31 mm voxel resolution).

MRI experiments on msP rats were performed on a 7 T scanner (Bruker, BioSpect 70/30, Ettlingen, Germany) using a receive-only phase array coil with integrated combiner and preamplifier in combination with an actively detuned transmit-only resonator. T_1 -weighted images were acquired using a multi-slice multi-echo (MSME) pulse sequence (TR = 300 ms, TE = 14 ms, averages = 8, field of view FOV = 32 x 32 and 9 horizontal slices of 0.5 mm thickness, matrix size = 256 x 256, resulting in 0.125 x 0.125 x 0.5 mm voxel resolution).

Data analysis

All MRI datasets were analyzed at the Neuroscience Institute (CSCI-UMH) following the same pipeline. The MRI images were converted to Analyze 7.5 format and scaled up by a factor of 10. All images were preprocessed with custom-developed MATLAB functions (version R2011a). In brief, brain-extracted (FSL BET, Oxford Centre for Functional Magnetic Resonance imaging of the Brain, London, UK) (Smith, 2002) T_1 -weighted images were co-registered and further registered to a stereotaxic rat brain MRI template (Schwarz et al., 2006) by a 12-parameter affine transformation using the FSL/FLIRT tool (Jenkinson et al., 2002). The template is co-registered to a digitized atlas (Paxinos and Watson, 2007), which enabled atlas-based generation of region-of-interest (ROI) masks for detailed anatomical analysis. The resulting images were smoothed to improve signal-to-noise

ratio using a Gaussian kernel with $4 \times 4 \times 4$ mm FWHM for both msP and AA. For identifying the brain regions in which activation in the alcohol-drinking groups differed from the water-drinking control group, voxel-wise independent t-tests were performed in SPM8 (www.fil.ion.ucl.ac.uk/spm/). For pump infusion experiments in AA and msP rats, the arbitrary significance threshold was set to $P < 0.01$ (uncorrected) for individual voxels, and then a cluster size threshold was applied to correct for multiple comparisons at $P < 0.05$. The cluster size threshold value was determined using Monte Carlo simulation (54 and 13 voxels for AA and msP rats, respectively). For the acute i.p. MnCl_2 administration experiment in msP rats, a significance level of $P < 0.05$ corrected for multiple comparison was defined (cluster size of 86 voxels defined by Monte Carlo simulation). The same data were also tested for lower or higher p-values. The thresholded statistical maps were overlaid on a common anatomical template.

STATISTICS

To test the possible toxic effects of MnCl_2 infusion on alcohol drinking, daily alcohol intake was testing using one-way repeated measures analysis of variance (ANOVA) in both rat strains. In addition, the strains were compared using a two-way (strain, time) ANOVA, with repeated measures on time. In the repeated measures ANOVAs, assumption of sphericity was tested with Mauchly's sphericity test. If sphericity was violated, the degrees of freedom of the F-distribution were corrected with the Greenhouse-Geisser procedure. The level for statistical significance was set at $p < 0.05$.

RESULTS

Behavioral effects of MnCl_2 infusion

At the baseline before implantation of MnCl_2 -containing osmotic minipumps, AA and msPrat strains reached similar levels of daily alcohol consumption, approximately 4-5 g/kg body weight/day. Manganese administration produced no observable behavioral alteration, including locomotor activity. One-way ANOVA indicated that either the MnCl_2 infusion, the surgical procedures, or both altered alcohol drinking in the AA rats ($F_{2,33, 21.0} = 4.19$, $p = 0.025$). The reduction in alcohol intake was transient, reaching statistical significance during the second and third infusion days. By the fourth day, the intake returned to the baseline (Fig. 1). No significant changes in alcohol drinking were found in the msP rats ($F_{2,45,31.84} = 0.37$, $p = 0.74$) and the comparison of AA and msP rats revealed no differences between them ($F_{1,22} = 0.53$, $p = 0.47$).

Functional mapping of brain activity during alcohol drinking in AA rats

Statistical parametric maps presented in Fig. 2 show statistically significant ($P < 0.05$, corrected) increase of T_1 -signal intensity in AA alcohol drinking rats compared to the water-drinking control animals. No decreases in T_1 -signal were found statistically significant. Enhanced T_1 intensity reflects accumulation of Mn^{2+} into brain tissue and therefore suggests increased functional brain activation. In the forebrain, alcohol-induced activation was observed bilaterally in the nucleus accumbens, anterior insula, regions of the piriform cortex, and medially in the anterior cingulate cortex. The striatal activation cluster belonged to a continuum that extended to the ventral pallidum, preoptic area, bed nucleus of the stria terminalis, anterior hypothalamus, and the perifornical area of lateral hypothalamus. Both dorsal and ventral regions of hippocampus displayed activation. In the midbrain, activation was found in the substantia nigra and periaqueductal grey. The largest percentwise activation was seen in the ecto- and perirhinal cortices, as well as the temporal association cortex, that consisted of a unilateral cortical activation cluster (Fig. 4). Other cortical areas displaying activation included the primary and secondary motor and somatosensory cortices.

Functional mapping of brain activity during alcohol drinking in msP rats

Statistical parametric maps presented in Fig. 3 show statistically significant ($P < 0.05$, corrected) increase of T_1 -signal intensity in msP alcohol drinking rats compared to the water-drinking control group. As for AA rats, no decreases in T_1 -signal were found. Activity increases revealed by osmotic mini-pump infusion of $MnCl_2$ were largely confined to prefrontal cortical regions including the cingulate, prelimbic, infralimbic, peduncular and orbitofrontal cortex, as well as the insular cortex. Several olfactory areas including the olfactory bulb, accessory olfactory bulb, anterior olfactory nucleus, tenia tecta, and piriform cortex were identified. Activity increases in subcortical regions were mainly restricted to the nucleus accumbens.

DISCUSSION

Our present data demonstrate the utility of MEMRI for capturing brain activation patterns accumulated over several days in freely behaving animals, in agreement with previous studies using this imaging technique. Most importantly, we showed remarkable differences in brain activation between two rat lines, AA and msP rats, selected for high alcohol drinking and displaying identical alcohol drinking levels in the current experiments. The level of voluntary alcohol drinking by these rat lines has previously been shown to induce psychomotor stimulatory, antidepressant, and anxiolytic actions (Paivarinta and Korpi, 1993; Colombo et al., 1998; Ciccocioppo et al., 2006). Furthermore, prolonged alcohol consumption lead to persistent brain activation changes during early abstinence (Dudek et al., 2015), and to white and grey matter integrity alterations that paralleled those found in human alcoholics (De Santis et al., 2019; De Santis et al., 2020). Together, these behavioral and neural consequences of high and prolonged alcohol drinking in these lines model several important features of the trajectory of human AUD and suggest that the brain imaging data obtained from these models could have translational value.

Differential Mn^{2+} accumulation between the alcohol-exposed and control groups can most likely be attributed both to the direct neurochemical effects of ingested alcohol and the activity of brain circuits underlying the incentives to drink alcohol. As previously described, administration of MnCl_2 with continuous systemic infusion produced no toxicity and only minor behavioral alterations (Eschenko et al., 2010b; Eschenko et al., 2010a; Dudek et al., 2015). Together, these data suggest that MEMRI is a versatile approach for studying various alcohol-related behaviors, and the ease of obtaining enhanced T1 contrasts allows comparison of data across different imaging platforms. Additional benefits of MEMRI for mapping brain activity include its independency from the nonlinearities introduced by neurovascular coupling in imaging techniques such as fMRI with BOLD contrast, and the high spatial resolution provided by the robust MR sequences used for T_1 -weighted imaging. The use of anesthesia during MEMRI can be considered a confounding factor. On the other hand, isoflurane reduces neuronal excitability and it is not likely that isoflurane during a 1-hour imaging session could significantly alter the neuronal Mn^{2+} concentrations accumulated over several days. When Mn^{2+} is administered as continuous infusion as in the present experiments, the temporal resolution of the method is poor. However, our aim was not to map acute effects of alcohol ingestion, but to characterize the networks repeatedly activated during long-term alcohol drinking. MEMRI has been increasingly exploited for identifying neural circuits associated with specific behaviors in similar experimental settings (Eschenko et al., 2010b; Gildish et al., 2012; Bangasser et al., 2013; McGuire et al., 2013; Laine et al., 2017; Yang and Li, 2020).

Although our most striking finding was the engagement of widely different circuits in AA and msP rats, we also saw important overlaps in regional brain activity. Activation of common neural substrates by alcohol in these lines was expected because similar neurochemical actions of alcohol have been found across various genetic models of alcohol-drinking (McBride and Li, 1998; Ciccocioppo et al., 2006; Sommer et al., 2006). One of these structures was the nucleus accumbens that has long been implicated in the reinforcing effects of drugs of abuse, including alcohol (Koob et al., 1998). The nucleus accumbens activation revealed by MEMRI is also in line with previous

attempts to map global brain activation by alcohol using either deoxyglucose metabolic mapping (Porrino et al., 1998) or c-Fos immunohistochemistry (Bachtell et al., 1999; Hansson et al., 2008; Vilpoux et al., 2009). Furthermore, either experimenter-administered or self-administered alcohol increased extracellular dopamine release in the nucleus accumbens both in the AA and sP rats, the parental strain of msPs (Nurmi et al., 1998; De Montis et al., 2004). The ventral striatal activation has been consistently associated with presentation of alcohol or alcohol-associated cues also in humans (Myrick et al., 2004; Filbey et al., 2008; Gilman et al., 2008; Vollstadt-Klein et al., 2020). Other brain regions comprising the basic motivational circuit emerging from human imaging include the orbitofrontal/medial aspects of the prefrontal cortex and insular cortex, both found to be activated also in AA and msP rats in the present study. The insula has been implicated in interoceptive effects of drug taking, and its contribution to drug-related responses has been demonstrated both in humans and animal models including the AA line (Contreras et al., 2007; Naqvi and Bechara, 2009; Drouman et al., 2015; Haaranen et al., 2020). Interestingly, ventral striatal and insula activation to alcohol cues was more amplified in high drinking than low drinking humans (Ihssen et al., 2011), and the projection from the insula to nucleus accumbens is involved in alcohol consumption both in rodents and human alcoholics (Seif et al., 2013; Grodin et al., 2018; Jaramillo et al., 2018). Collectively, the activation of the common brain regions in AA and msP rats, including the nucleus accumbens, medial prefrontal cortex and insula, is an expected effect of alcohol cues and the presence of alcohol in the body, but it may also reflect the motivational processes driving alcohol drinking in these animals.

Apart from these overlapping structures, the markedly different overall activation patterns in these lines could be related to their genetic background and the resulting behavioral and neurochemical differences. In the AA rats, the lack of effect by mesolimbic dopamine depletion on alcohol drinking suggests that alcohol reinforcement could be mediated also through other systems in these rats (Koistinen et al., 2001). The major activation cluster found in the bed nucleus of the stria terminalis (BNST) and various hypothalamic nuclei supports this view. The BNST has been strongly implicated

in stress responses, but its nuclei are also closely connected with the key components of circuitry that mediates positive reinforcement, including VTA, ventral striatum, and hypothalamic nuclei (Wills and Winder, 2013). Various transmitter systems of the BNST, such as dopamine and GABA, have been associated with alcohol reinforcement (Hyytia and Koob, 1995; Carboni et al., 2000; Eiler et al., 2003). Also, more chronic alcohol exposures (Olive et al., 2002) or alcohol-associated environmental cues (Hill et al., 2007) altered BNST functioning, further demonstrating its importance for alcohol-motivated behavior. Since the BNST is bidirectionally connected with hypothalamic nuclei (Alheid and Heimer, 1988), the observed activation cluster suggests particular relevance for the BNST and hypothalamus for mediating alcohol reinforcement in AA rats. Various neuropeptides in hypothalamic regions regulate alcohol drinking in a manner dissociable from a generalized appetitive motivation control, including orexin-containing neurons localized in the dorsomedial, perifornical, and lateral hypothalamus (Peyron et al., 1998; Walker and Lawrence, 2017), as well as oxytocin-containing neurons in the medial hypothalamus (Peters et al., 2017; Hansson et al., 2018; Tunstall et al., 2019). Therefore, the hypothalamic activation cluster in AA rats could pinpoint an important, previously unrecognized region for mediating voluntary alcohol drinking in these rats.

In sharp contrast to AA rats displaying widespread subcortical and cortical activation, the pattern found in msP rats was highly restricted to the rostral and ventral parts of the striatum and medial prefrontal cortex. These differences are probably not due to the lower Mn^{2+} dose used in msP rats, because the average signal enhancement was slightly higher in msP rats and the differences in activation patterns were qualitative. The strong activation of the medial prefrontal cortex (mPFC) including the infralimbic and prelimbic areas seems therefore genuine in msP rats, and a property that this line shares with the post-dependent model, in which rodents are exposed to high blood alcohol levels during chronic intermittent ethanol (Meinhardt and Sommer, 2015). Prolonged alcohol exposure caused a profound reprogramming in the mPFC, leading to molecular dysregulation (Heilig et al., 2017) and alterations in activity and connectivity (Scuppa et al., 2020)

that probably contribute to the alcohol-dependent phenotype. Similar alterations appear to preexist in the msP line (Bjork et al., 2010; Bifone et al., 2019). Both the infralimbic and prelimbic cortices have been implicated in fear and drug-seeking, with a dorsal-ventral distinction. Thus, the prelimbic cortex has been suggested to drive the expression of fear and drug seeking, whereas the more ventral infralimbic cortex promotes the extinction of conditioned fear and drug seeking (Peters et al., 2009). Given these seemingly competing roles of the dorsal and ventral medial prefrontal cortex in fear and drug seeking, the functional significance of the observed activation in both areas is not clear. However, it could be hypothesized that activation of the infralimbic prefrontal cortex by alcohol drinking in msP rats may be related to inhibition of the innate anxiety-like behavior in this line (Ciccocioppo et al., 2006; Pfarr et al., 2015), whereas the prelimbic cortex activation mediates alcohol-seeking behavior. The infralimbic cortex activation inhibits the central amygdala (Quirk et al., 2003), which is in line with a previous finding that alcohol drinking decreased the anxiety-associated overactivity of corticotropin-releasing hormone receptor 1 (CRH-R1) in the amygdala in msP rats (Hansson et al., 2006; Hansson et al., 2007). In the present experiments, however, no evidence for decreased activity either in msP or AA rats was found. This may reflect the limited ability of MEMRI to detect deactivation, as the enhanced T1 signals are produced by Mn^{2+} entry associated with neuronal activation. In further support for the involvement of the infralimbic cortex in alcohol effects, chronic intermittent alcohol exposure attenuated mGluR2/3 autoreceptor function and NMDA-receptor mediated currents in the pyramidal neurons of the infralimbic cortex, which was related to persistent escalation of alcohol self-administration and deficient fear extinction (Holmes et al., 2012; Meinhardt et al., 2013).

Laboratory-specific differences in hardware and imaging parameters could contribute to either the quality of the data or the patterns of brain activation and can therefore be considered a limitation of this study. However, contrast enhancement in the T_1 -weighted MR images produced by Mn^{2+} accumulation is very robust, and it is not likely that minor differences in sequence parameters could have contributed to the differential activation pattern in these rat strain. In addition, we saw more

widespread brain activation in the AA rats with a lower 4.7T field than the restricted frontal pattern in the msP rats imaged using a higher 7T field strength and a higher SNR. In both cases, the spatial localization of activation was detailed and region-specific, suggesting that we captured qualitatively different brain activation patterns in these two rat strains.

Taken together, the patterns of functional brain activation induced by voluntary alcohol drinking in AA and msP rats appear to recapitulate the pivotal elements of the motivational circuits underlying alcohol and drug taking suggested by previous animal and human studies. However, the intriguing novel finding was that despite similar alcohol consumption levels, the neural circuits activated by alcohol drinking were conspicuously different in these rat lines. One way of interpreting these differences is to relate them to the co-segregated behavioral traits, i.e., risk-taking and impulsivity in the AA and anxiety-like behavior in the msP rats, and to claim that the observed dissimilarity in functional circuits reflects innately different behavioral processes and states driving alcohol drinking. The current data may also suggest potential neuroanatomical substrates for the heterogeneity seen in human alcoholic patients. The brain networks driving alcohol drinking in the animal models could be subjected for further circuit-level interrogation for pharmacological treatment effects (Dudek et al., 2016). Comparison of those data with respective human imaging outcomes could facilitate our understanding of dimensions of behavior and identify targets for treatment (Heilig et al., 2016; De Santis et al., 2019; Heilig et al., 2019; De Santis et al., 2020). Such translational framework for AUD is needed to fully implement recent proposals to transcend categorical psychiatric diagnosis such as the Research Domain Criteria (RDoC) and Addictions Neuroclinical Assessment initiatives (Kwako et al., 2017).

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FIGURE LEGENDS

Figure 1. Effects of the seven-day MnCl_2 infusion with osmotic minipumps on alcohol consumption in msP ($n = 14$) and AA rats ($n = 10$). Data present mean ($\pm$ SEM) in each group. * $P < 0.05$, significantly different from the baseline (BL), defined as the mean of the previous seven days (Dunnett's test).

Figure 2. Brain activity maps during alcohol drinking in AA rats. T_1 -weighted MRI signal enhancement was found in alcohol drinking rats compared to water-drinking control animals. Only signal enhancements were found (alcohol > water). Shown are coronal (A), sagittal (B) and horizontal (C) functional MEMRI maps threshold at $p < 0.05$ (two-tailed t-test, corrected, $n = 10/\text{group}$) and overlaid on representative T_1 -weighted images. Color code represents t-values. Numbers in the left-bottom corner of each image indicate the positions of the sections from bregma in millimeters. Abbreviations: Acb, nucleus accumbens; Ins, Insular cortex (mainly agranular); Cg, cingulate cortex; BNST, bed nucleus of the stria terminalis; vHC, ventral hippocampus; LH, lateral hypothalamus; LS, lateral septum; Pir, piriform cortex; CeA, central amygdaloid nucleus; AH, anterior hypothalamus.

Figure 3. Brain activity maps during alcohol drinking in msP rats. T_1 -weighted MRI signal enhancement was found in alcohol drinking rats compared to water-drinking control animals. Only signal enhancements were found (alcohol > water). Shown are coronal (left column) and horizontal (right column) MEMRI maps obtained using osmotic minipumps for manganese infusion ($p < 0.05$, two-tailed t-test, corrected, $n = 14/\text{group}$). Color code represents t-values. Numbers in the right-bottom corner of each image indicate the positions of the sections from bregma in millimeters. Abbreviations: PrL, prelimbic cortex; IL, infralimbic cortex; AON, anterior olfactory nucleus; Ins, Insular cortex (mainly agranular); Acb, nucleus accumbens; Cg, cingulate cortex; CPu, caudate putamen.

Figure 4. Distinct brain networks are activated by alcohol drinking in AA and msP rats. Brain regions with enhanced manganese accumulation in AA (red) and msP (blue) rats are co-registered and overlaid on corresponding sagittal (A) and coronal (B-C) slices of the Paxinos and Watson (2007) rat

brain atlas. Statistical thresholds are the same used in Figs 2 and 3. Dotted lines in (A) mark the position of the coronal slices in (B) and (C), and *vice versa*. The combined representation of AA and msP MEMRI maps for the complete brain volume can be seen in Movie 1. (D) Histogram representation of the volume of specific brain regions activated by alcohol drinking (in % of the atlas-defined total volume of each region) in AA (red) and msP (blue) rats. The list of brain regions was composed both based on size of the activated areas in msP and AA rats and the proposed neurocircuitry of alcohol effects (Noori et al., 2012). Abbreviations: OB, olfactory bulb; AON, anterior olfactory nucleus; PirC, piriform cortex; EndP, endopiriform cortex; OC, orbitofrontal cortex; FrA, frontal association cortex; PrL, prelimbic cortex; IL, infralimbic cortex; Ins, insula; Cg, cingulate cortex; M1, primary motor cortex; M2, secondary motor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; HC, hippocampus; PRh, perirhinal cortex; EctC, ectorhinal cortex; TeA, temporal association cortex; Acb, nucleus accumbens; CPu, caudate putamen; VP, ventral pallidum; Amy, amygdala; Bed, bed nucleus of stria terminalis; PO, preoptic area; H, hypothalamus; SN, substantia nigra; PAG, periaqueductal grey.

Movie 1. Combined representation of AA and msP MEMRI maps ($p < 0.05$, corrected) across the complete brain volume. Brain regions with enhanced manganese accumulation in AA (red) and msP (blue) rats are co-registered and overlaid on an anatomical image. Each frame of the movie represents one coronal slice.

Figure 1

Figure 2

Figure 3.

Figure 4.