NON-HUMAN PRIMATE MODELS OF PSYCHIATRIC DISORDERS

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NON-HUMAN PRIMATE MODELS OF PSYCHIATRIC DISORDERS

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EDITORIAL
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Editorial: Non-human Primate Models of Psychiatric Disorders

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Keywords: primate, psychiatric disorder, animal models, behavior, translational studies

Editorial on the Research Topic

Non-human Primate Models of Psychiatric Disorders

As our civilization faces the complex social and health challenges of the twenty-first century, the quest for scientific progress seems more critical than ever before. The field of Biomedical research, in particular, is required to balance this pressing demand for knowledge with ethical constraints on both human and animal research. This issue raises fierce debates on the relevance of animal models for the advancement of science and society well-being, going as far as questioning if current animal research is still ethical, or even necessary. Although the reasoning for pursuing ethical experimentation on animal subjects is obvious for researchers in the field, the public discourse is often obscured by science illiteracy and misinformation. Ethical debate over the justification of animal research is particularly controversial in the case of non-human primates (NHP).

Despite steady progress in computational techniques, *in-silico* models, and transgenic rodent models, all of these approaches are still far from replacing NHPs. Translational methods for Psychiatric research should produce results which are, at the very least, predictive of one or more clinical outcomes. In this regard, NHPs are by far best suited to model psychiatric symptoms. The phylogenetic proximity to humans translates into more similar physiological and behavioral profiles, social structures, and developmental progress. For instance, NHPs are reliably used in addiction studies [see Maior (2011)] as they show reactions to those observed in humans during abstinence (Weerts et al., 2007). Also, very specific and complex responses, such as hallucinatory behavior, can be used as behavioral parameters in NHP (e.g., Ellison et al., 1981; Castner and Goldman-Rakic, 2003).

In this sense, the present topic gathered a small but very representative sample of studies which underscores the importance of NHP research for Psychiatry and Biomedicine. It includes diverse techniques and strategies to explore neurobiological disorders, ranging from stress and anxiety to schizophrenia and addiction. Natural drives and experimental evidence reveals marmosets as hitherto unappreciated models for research on psychiatric disorders related to stress de Sousa et al., while Novak and Meyer evaluate the existing evidence and future perspectives on marmoset models of depression, presenting a detailed review of self-injury behavior and discussing the prospects of this model for non-suicidal self-injury in human patients. Feng et al. reviewed the state-of-the-art regarding models of autism and their relationship with sleep disorders. In order to elucidate physiological aspects of addiction-related behaviors, Daddaoua et al. performed a detailed behavioral testing of decision-making strategies that underlie contingency management in macaques, bearing important perspectives for future addiction treatment in humans. Rowland et al. used magnetoencephalography in macaques to detail resting-state brain networks prior and post

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exposure to alcohol. Based on these data, they propose specific network characteristics which could predict high-risk of drinking phenotype. Costa et al. analyzed the feasibility of a translational model of schizophrenia employing visual illusions to probe the effects of glutamatergic antagonism. By means of single unit electrophysiological recordings, Dinh et al. explored the role of the prefrontal cortex in the processing of threatening stimuli and put forward a working hypothesis of how this structure may be involved in the origin of ophidiophobia. Waguespack et al. used intracerebral infusions in awake macaques to delineate the differences in brain circuitry related to sensory-gated responses between rodents and primates. Finally, Labuguen et al. present an important refinement to behavioral analyses of primates by providing open dataset of macaques which can be used for improving neural networks in motion capture tasks.

These studies outline the breadth of NHP research, and offer a valuable snapshot of how current primate research, using both classical and state-of-the-art techniques, continue to yield valuable insights into pathophysiological aspects of psychiatric conditions in the 2020's.

AUTHOR CONTRIBUTIONS

RM wrote initial draft. HN and FC edited and presented the final form. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Inhibition of the Deep and Intermediate Layers of the Superior Colliculus Disrupts Sensorimotor Gating in Monkeys

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The deep and intermediate layers of the superior colliculus (DLSC) respond to visual, auditory, and tactile inputs and act as a multimodal sensory association area. In turn, activity in the DLSC can drive orienting and avoidance responses—such as saccades and head and body movements—across species, including in rats, cats, and non-human primates. As shown in rodents, DLSC also plays a role in regulating pre-pulse inhibition (PPI) of the acoustic startle response (ASR), a form of sensorimotor gating. DLSC lesions attenuate PPI and electrical stimulation of DLSC inhibits the startle response. While the circuitry mediating PPI is well-characterized in rodents, less is known about PPI regulation in primates. Two recent studies from our labs reported a species difference in the effects of pharmacological inhibition of the basolateral amygdala and substantia nigra pars reticulata (SNpr) on PPI between rats and macaques: in rats, inhibition of these structures *decreased* PPI, while in macaques, it *increased* PPI. Given that the SNpr sends direct inhibitory projections to DLSC, we next sought to determine if this species difference was similarly evident at the level of DLSC. Here, we transiently inactivated DLSC in four rhesus macaques by focal microinfusion of the $GABA_A$ receptor agonist muscimol. Similar to findings reported in rodents, we observed that bilateral inhibition of the DLSC in macaques significantly disrupted PPI. The impairment was specific to the PPI as the ASR itself was not affected. These results indicate that our previously reported species divergence at the level of the SNpr *is not* due to downstream differences at the level of the DLSC. Species differences at the level of the SNpr and basolateral amygdala emphasize the importance of studying the underlying circuitry in non-human primates, as impairment in PPI has been reported in several disorders in humans, including schizophrenia, autism, and PTSD.

Keywords: macaque, superior collicullus, acoustic startle reflex, pre-pulse inhibition (PPI), GABAA receptors, pharmacological inhibition, sensorimotor gating, muscimol

INTRODUCTION

Sensorimotor gating—the ability to shift attentional resources to salient stimuli—is often operationalized and studied by assessing pre-pulse inhibition (PPI) of the acoustic startle response (ASR). In mammals the ASR is a rapid, reflexive contraction of skeletal, facial, and body muscles in response to a sudden, intense auditory stimulus (Davis et al., 1982; Koch and Schnitzler, 1997) and is conserved across many species, including rats, mice, guinea pigs, cats, dolphins, non-human primates, and humans (Saitoh et al., 1987; Wu et al., 1990; Geyer, 1999; Dawson et al., 2008; Dehmel et al., 2012; Saletti et al., 2014; Götz et al., 2020). PPI refers to the attenuation of the ASR when a subject is exposed to a low-intensity auditory "prepulse" prior to a startle-inducing auditory "pulse" (Geyer et al., 1990). PPI has been reported to be disrupted in several neuropsychiatric disorders, including schizophrenia, obsessive compulsive disorder, and post-traumatic stress disorder (PTSD) (Grillon et al., 1996; Kohl et al., 2013).

In rodents, both permanent lesions and transient pharmacological manipulation of basal ganglia input nuclei (e.g., nucleus accumbens), relay nuclei (e.g., ventral pallidum), and output nuclei (e.g., the substantia nigra pars reticulata; SNpr) disrupt PPI (Wan and Swerdlow, 1993; Kodsi and Swerdlow, 1997; Kretschmer and Koch, 1998; Forcelli et al., 2012; Aguilar et al., 2018). In a parallel study employing transient pharmacological inhibition of the SNpr in both rodents and non-human primates (Aguilar et al., 2018), we recently reported a surprising divergence of effect—while infusion of the $GABA_A$ receptor agonist muscimol into the SNpr *disrupts* PPI in rodents, we found that the same manipulation *potentiates* PPI in rhesus macaques, suggesting that the contribution of basal ganglia circuits to PPI may differ between the rodent and primate brain.

The superior colliculus (SC), a major downstream target of basal ganglia efferent output, has a well-established role in PPI in rodents. Lesions of the SC disrupt PPI (Fendt et al., 1994), while transient blockade of $GABA_A$ receptors in the SC augments PPI (Fendt et al., 1994; Fendt, 1999). Moreover, focal blockade of glutamate receptors in the deep and intermediate layers of the SC (DLSC) impairs PPI (Ding et al., 2019) and focal microstimulation of the DLSC can act as a pre-pulse and suppress auditory-evoked startle (Li and Yeomans, 2000).

The DLSC receive multimodal sensory input (visual, auditory, tactile), and coordinate eye, head, and whole-body movements to stimuli. Stimulation of the DLSC can evoke both orienting (e.g., saccades) and escape-like responses in rodents (McHaffie and Stein, 1982; Dean et al., 1989; Comoli et al., 2012), cats (Stein et al., 1980), and non-human primates (Hikosaka and Wurtz, 1983, 1985; DesJardin et al., 2013). In rodents, approach and avoidance behaviors display a medio-lateral and rostro-caudal topography (Dean et al., 1989; Comoli et al., 2012). By contrast, in non-human primates we have previously reported that activation of both medial and lateral superior colliculus produces avoidance responses (DesJardin et al., 2013). Consistent with this role for the SC in defense, avoidance, and threat detection, recent neuroimaging studies have revealed increased activity in the SC in individuals with PTSD both in response to prolonged eye contact (Steuwe et al., 2014) and in response to subliminal presentation of threat-related stimuli (Terpou et al., 2019). This is particularly interesting given that PPI and acoustic startle have been reported to be disrupted in PTSD (Ornitz and Pynoos, 1989; Butler et al., 1990; Grillon et al., 1996; Morgan et al., 1996; Shalev et al., 2000; Pineles et al., 2016). Specifically, two studies from Vietnam War veterans with combat-related PTSD reported significantly increased startle response and disrupted PPI, respectively (Butler et al., 1990; Grillon et al., 1996). These effects extend beyond combat-related trauma and are not specific to adults, nor are they dependent on sex. One study in children with PTSD and a second in women with PTSD both found a significant loss of PPI (Ornitz and Pynoos, 1989; Pineles et al., 2016).

Given (1) the recent reports of midbrain (superior colliculus) involvement in PTSD in clinical populations, and (2) that the DLSC are a primary efferent target of the SNpr, which we have found to differ in function with respect to PPI in rodents and primates, we sought to determine if the previously described species difference in the effect of SNpr inhibition on PPI was similarly present at the level of the DLSC. To address this, we microinjected a $GABA_A$ receptor agonist, muscimol, into the DLSC of four macaque monkeys and assessed acoustic PPI through measurement of the whole-body ASR.

MATERIALS AND METHODS

Experimental Design

To evaluate the role of the DLSC in sensorimotor gating in rhesus macaques, we measured PPI of the whole-body ASR of monkeys seated in a primate chair. Four rhesus macaques were implanted with cranial microinfusion platforms and infused bilaterally with muscimol into the DLSC for transient, reversible inactivation of the target region or bilateral injection of saline as a control. Manipulations were performed on a within-subject basis on a randomized treatment schedule.

Subjects

Four male, rhesus macaques (*Macaca mulatta*) were used in this study (NO, SL, RE, and OD). At the age of 2–3 years, they were procured from AlphaGenesis and transferred to Georgetown University, where all experimental procedures were conducted. Monkeys were pair-housed within two joined individual cages (size, 61 × 74 × 76 cm each). The monkeys were housed in a room with a regulated 12 h light/dark cycle and maintained on a primate lab diet (Purina Mills, catalog #5049), supplemented with fresh fruit and vegetables. Water was available *ad libitum* in the home cage.

Care and housing of the monkeys at the Georgetown University Division of Comparative Medicine met or exceeded the standards as stated in the Guide for Care and Use of Laboratory Animals [National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011], Institute for Laboratory Animal Research recommendations, and AAALAC International accreditation standards. The study was conducted under a protocol approved by the Institutional Animal Care and Use Committee at Georgetown University.

The present experiments began after the animals were extensively socialized and behaviorally trained (including chair training), which continued until the age of about 4 years. At the time of testing, animals ranged from 4–6 years old and weighed between 5 and 9 kg. In addition to the experimental procedures described here, all subjects were trained on various cognitive tasks administered at the Wisconsin General Testing Apparatus; the tasks included the Hamilton Search task and a task that tested reactivity to emotionally salient stimuli (Elorette et al., 2020). As part of those experiments, some animals received drug infusions in the basolateral amygdala (NO, SL, RE), SNpr (NO, SL), parahippocampal cortex (OD, RE, SL), hippocampus (OD, RE, SL), periaqueductal gray (RE, SL), and pulvinar (SL). Infusions into the DLSC for PPI experiments occurred toward the conclusion of all other experimentation and overlapped most prominently with injections into the hippocampus and parahippocampal cortex. We do not anticipate that the prior or concurrent infusions influenced the data for this project, as all of our animals exhibited the expected pattern of increasing PPI as a function of pre-pulse intensity after saline/control infusions. Disruptions to PPI were only observed following infusion of muscimol to the DLSC.

Implantation of Drug Infusion Platform and Site Verification

The monkeys were implanted with a stereotaxically positioned chronic infusion platform, which enabled us to target specific sites within the DLSC based on the coordinates assessed by structural magnetic resonance imaging (MRI) scans. For the pre- and post-operative MRI and the surgery, we followed procedures as described in detail in our previous studies (Forcelli et al., 2016, 2017a,b; Wellman et al., 2016; Aguilar et al., 2018). Briefly, the infusion platform was implanted under anesthesia and aseptic conditions (Wellman et al., 2005; Forcelli et al., 2016), followed by a post-operative regimen of analgesics and antibiotics determined in consultation with the facility veterinarian. Postoperatively, each monkey received at least one T1-weighted structural MRI scan (0.75 × 0.75 mm in-plane resolution, 1 mm slice thickness) intended to obtain coordinates for infusions in the DLSC. Tungsten micro-electrodes (FHC; Bowdoin, ME), which were visible on the scan, were used to determine the precise coordinates as described previously (Holmes et al., 2012). **Figure 1B** shows the placement of a tungsten microelectrode at the dorsal border of the SC in two subjects. A figure of the infusion platform and telescoping cannula can be found in Wellman et al. (2005).

Intracerebral Drug Infusions

To transiently inactivate the DLSC (for infusion sites, see **Figure 1A**), 9 nmol of the $GABA_A$ receptor agonist muscimol (Sigma-Aldrich) in a volume of 1 ul (9 mM) was infused bilaterally at rate of 0.2 ul/min under aseptic conditions as previously described (Forcelli et al., 2017a). In our previously published studies, our lab found that injection of 1 μl of the contrast agent gadolinium (5 mM in saline) resulted in an area hypersignal of ∼3 mm in diameter (DesJardin et al., 2013; Forcelli et al., 2014), suggesting a sphere of drug spread of ∼3 mm an hour after infusion.

The entire infusion procedure lasted 10–15 min, and animals began the PPI procedure ∼5–10 min after the microinfusion was completed. At least 48 h elapsed between drug treatments in an individual subject. Control infusions consisted of either microinjection of an equivalent volume of sterile saline or a "sham" infusion. For sham infusions, all procedures were followed, but no cannula was lowered into place; sham infusions were included to minimize the number of brain penetrations.

Across subjects, we found that sham infusions and saline infusions produced near-identical results. We tested for a difference between sham and saline infusions using a mixed-effects model with pre-pulse intensity and treatment (sham or saline) as fixed factors and monkey and test session as random effects. We found neither a main effect of treatment ($F_{1, 14.36} = 0.489$, $P = 0.496$) nor a treatment-by-pre-pulse intensity interaction ($F_{2, 34} = 0.036$, $P = 0.964$), and thus collapsed across these conditions for subsequent analyses. Sham infusions were performed in all animals; saline infusions were performed in RE, OD, and SL.

The number of infusions per animal ranged from 1 to 5 for muscimol and 2 to 6 for control. Variability in infusion number is due to loss of the cranial implant, as these were typically the last studies planned for each animal. The number of infusions per animal is shown in **Table 1**. In addition to the listed infusions, we dropped one session control and one muscimol session from Animal OD, and one session from Animal RE as they were outliers on a within-subject basis (ROUT test, $Q = 0.1\%$).

PPI Task Set-Up

PPI testing was conducted using an apparatus modified from that described by Winslow et al., 2002 and was conducted essentially as we have previously described (Aguilar et al., 2018). Tests were conducted in a behavior room located next to the home cage room, in a sound attenuated chamber containing a primate chair (Crist Instruments Co.) attached to a platform sitting on a load cell. The chamber (60 × 114 × 80 cm) also contained a speaker (25 cm above the head) for administration of noise stimuli. The primates' whole-body startle movements were transmitted via a 50 kg load cell (Sentran LLC; YG6-B) located between the chamber floor and the primate chair platform. The load cell was connected to an amplifier which transmitted a signal to a Windows XP computer running the Startle Response software (Med Associates). Prior to experimentation, we calibrated the amplifier using a 10 kg weight and maintained this calibration setting across all animals. All animals weighed between 6 and 9 kg, so this single calibration was sufficient.

Animals were habituated to the apparatus during a training period of about 3 weeks. During the first week, animals were placed in the PPI chamber daily for sessions increasing by 5 min each day, from 5 to 25 min, with the door open and while receiving continuous positive reinforcement from the experimenter (e.g., grapes). During the second week, animals were placed in the chamber daily for the same increasing session durations. However, during this phase, the door was closed and animals were exposed to white noise (70 dB). Animals were

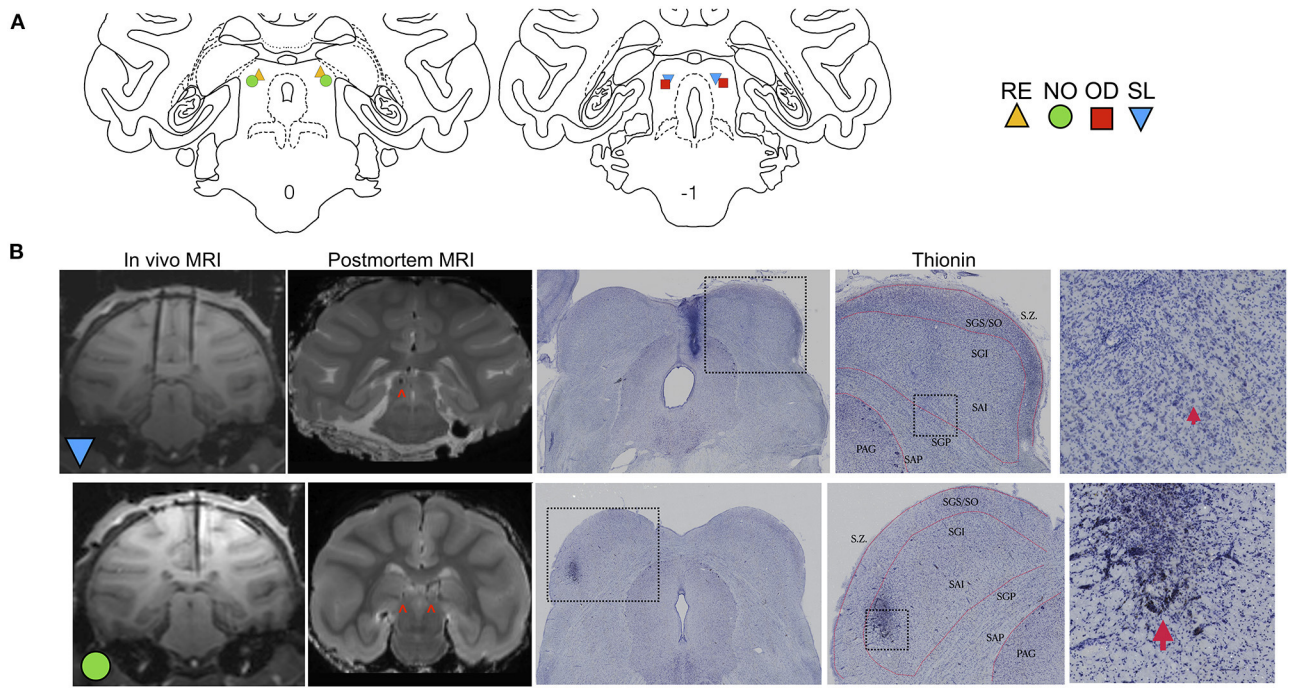

FIGURE 1 | Localization of DLSC infusion sites. **(A)** Coronal sections of a macaque brain atlas showing anterior DLSC (0.0 mm) and posterior DLSC (−1.0 mm). Symbols indicate locations of injection sites for each animal (SL, blue triangle; OD, red square; RE, yellow triangle; NO, green circle), determined from *in vivo* MRI (all animals) and confirmed by post-mortem MRI and histology (two animals). **(B)** *In vivo* MRI used for site localization, post-mortem MRI for site confirmation, and histology for site confirmation. In the *in vivo* images, tungsten microelectrodes can be seen dorsal to the DLSC in SL and NO. In the both post-mortem MRI images and histological images, arrows point to the cannula tracts resulting from microinfusion of muscimol or saline into the DLSC. Boxes outline magnified images in subsequent panels. Layers of the SC are outlined in red. SZ, stratum zonale; SGS/SO, stratum griseum superficiale/stratum opticum (superifical layers); SGI, stratum griseum intermedium; SAI, stratum album intermedium; SGP, stratum griseum profundum; SAP, stratum album profundum; PAG, periaqueductal gray. As shown, there is minimal tissue damage to the SC, and injections fell within the deep and intermediate (SGI/SAI/SGP/SAP) layers.

TABLE 1 | Number of injections performed in each subject.

Monkey	SL ▼	NO ●	RE ▲	OD ■
# of muscimol injections	5	1	4	3
# of Control injections	5 (1)	2	6 (2)	6 (2)

Number in parentheses indicate the number of saline infusions out of the total number of control injections per subject. Symbols correspond to those in ***Figures 1–3****.*

positively reinforced before and after door closure during this phase. During the third week, short (20 min) baseline sessions were run in which the animals were exposed to startling stimuli for the first time.

PPI Protocol

Each 50 min session consisted of a 3-min acclimation period with background noise (70 dB), 6 blocks of 3 randomized startling stimuli (90, 105, 110 dB; 40 ms pulse for OD, RE, and SL; 90, 100, 105 for NO, who was tested on an older version of the protocol), 15 blocks of 4 randomized trials containing pulse-alone (105 dB; 40 ms) and prepulse-pulse (pre-pulses: 4, 8, and 12 dB above background noise; 20 ms) trials, and 10 blocks of 3 randomized startling stimuli (90, 105, 110 dB; 40 ms pulse). Pulse alone trials across the whole session were used as a control for maintenance of startle amplitude, and blocks containing startling stimuli at the beginning and end were used to calculate habituation to startle.

During the prepulse-pulse trials an inter-stimulus interval (onset to onset) of 50 ms was used based on our prior study (Aguilar et al., 2018). The inter-trial interval ranged from 15–30 s, randomly selected for each trial. Startle amplitude was defined as the peak load cell output voltage over a 175-ms period beginning at the onset of the pulse stimulus.

Post-mortem MRI and Histology

SL, NO, and OD were perfused as previously described (Elorette et al., 2018). For *post-mortem* MRI analysis, the brains of three animals (SL, NO, and OD) were examined at high field strength (7 Tesla) on a Brucker Biospin Magnet using a Turbo-RARE pulse sequence, as previously described (Forcelli et al., 2016). Following MR imaging, brains were processed for localization of infusion sites, as we have previously described (Wellman et al., 2005; Gale et al., 2012; Forcelli et al., 2014). Representative photomicrographs and MR images are presented in **Figure 1B**.

Data Pre-processing

On a session-by-session basis for each monkey, we performed automatic outlier removal using the ROUT (robust regression

and outlier removal) test ($Q = 10\%$) in GraphPad Prism 8. We have found that the within-subject, within-session variability of startle responses is often larger in macaques than in comparable studies in rodents, and thus removed high amplitude outliers which reflect motion in the chair unrelated to the startle response. We select a permissive removal criterion to remove all large amplitude, artifactual responses. The mean number of dropped trials for control sessions was 7.6, the mean number of dropped trials for muscimol sessions was 8.4, typically 1 to 3 trials were excluded for each trial type (i.e., pulse alone and each pre-pulse intensity). By dividing each session into thirds and calculating the number of trials excluded as outliers for each block within each session, we found that average number of dropped trials per session did not differ as a function of block ($F_{2,6} = 0.81$, $p = 0.49$), treatment ($F_{1,3} = 0.51$, $p = 0.53$), nor did we detect a block-by-treatment interaction ($F_{2,6} = 0.87$, $p = 0.46$). Thus we concluded that (1) our treatment did not influence the number of dropped trials and (2) habituation effects were unlikely to have influenced the rate of dropped trials.

Following artifact/outlier removal, we calculated PPI. PPI was defined as [1-(startle amplitude on pre-pulse trials/startle amplitude on pulse alone trials)] × 100 as previously described (Winslow et al., 2002; Davis et al., 2008). Startle habituation was calculated as: [1-(mean response on post-test trials/mean response on pre-test trials)]*100 on a session-by-session basis.

Statistical Analysis

We used the MIXED procedure in SPSS (Version 25) for data analysis with Restricted Maximum Likelihood estimation and the Satterthwaite approximation for degrees of freedom calculations. GraphPad Prism 8 was used for figure preparation.

ASR on pulse-alone trials was analyzed using a mixed-effects model with treatment as a fixed effect and monkey and session as random effects. PPI data were analyzed using a multi-level marginal model in SPSS, with treatment and pre-pulse intensity as fixed effect factors. Note that we also analyzed these data using a mixed effects model with treatment and pre-pulse intensity as fixed effects and monkey and session as random factors. This produced a model with poorer fit (based on both Akaike information criterion and Bayesian information criterion metrics) than the marginal model, although the fixed effects and pairwise comparisons of the estimated marginal means produced similar results. For this model, we generated estimated marginal means and present the difference in estimated marginal means, along with 95% confidence intervals of the differences as a visual estimation of effect size. As deficits in PPI are often overcome at higher pre-pulse intensities, we planned *a priori* to compare PPI as a function of treatment within each pre-pulse intensity. The ASR curve on the pre-test and post-test trials was analyzed using a mixed-effects model with treatment, block (beginning of session vs. end of session), and noise amplitude (90, 105, 110 dB) as fixed factors and monkey and session as random effects. Startle habituation was analyzed using a mixed-effects model with treatment and noise amplitude as fixed effects and monkey and session as random effects.

RESULTS

Infusion site verification is shown in **Figure 1**. The position of electrodes from the *in vivo* scans closely correspond to the localization of the cannulae tracks from post-mortem MRI scans and histological reconstruction. Histological analysis confirmed localization of infusion cannulae to the DLSC, and not the superficial layers of superior colliculus. Representative MR images and photomicrographs are shown in **Figure 1B**. Sites were further confirmed using behavior from a parallel set of experiments with these subjects: unilateral activation of the DLSC at the same site using bicuculine methiodide (2.5 nmol) resulted in contralateral saccades and defensive vocalizations in SL, NO, and RE, consistent with prior reports (DesJardin et al., 2013).

The effects of muscimol infusion into the DLSC on PPI are shown in **Figure 2**. As expected, we found a main effect of pre-pulse intensity ($F_{2,87.2} = 24.229$, $p = 0.000000004$), with increasing pre-pulse intensity associated with higher levels of PPI in both control (linear regression, $R^2 = 0.1704$, $p = 0.0014$) and muscimol-infused conditions (linear regression, $R^2 = 0.2752$, p=0.0002). We also found a main effect of treatment ($F_{1,87.4} = 6.198$, $p = 0.014$), but no pre-pulse intensity-by-treatment interaction ($F_{2,87.2} = 2.113$, $p = 0.127$). The main effect of treatment (indicated by x) is evident in difference in estimated marginal means plot shown in **Figure 2**. *A priori*, we planned to compare drug within each level of pre-pulse intensity. The treatment effect was driven by a significant reduction in PPI when pre-pulse intensity was 4 dB above background ($p = 0.002$, Sidak corrected). No significant differences were found for pre-pulse intensities of 8 ($p = 0.525$, Sidak corrected) or 12 ($p = 0.563$, Sidak corrected) dB above background. The magnitude of the drug effect for each pre-pulse intensity is shown in the plot of differences in estimated marginal means in **Figure 2**.

A change in magnitude of the ASR (i.e., the response to pulse-alone trials without a prepulse) may contribute to or mask changes in PPI (Sandner and Canal, 2007; Shoji and Miyakawa, 2018). In rodents, lesions (Groves et al., 1974; Fendt et al., 1994) and pharmacological activation (Fendt, 1999) of the DLSC have been reported to have no effect on baseline startle. However, one report found *enhanced* baseline startle response after DLSC lesions in rodents (Tischler and Davis, 1983). Consistent with the majority of the existing literature, we found no effect of muscimol infusion in SC on baseline startle amplitude ($F_{1,7.4} = 1.053$, $p = 0.337$, mixed effects model, **Figure 3**). We did, however, note that the average startle amplitude for each of the four animals was numerically decreased after muscimol infusion.

We next compared the startle response curves as a function of noise amplitude during the pre- and post-test blocks (**Figures 4A,B**, respectively). We found a significant main effect of noise amplitude ($F_{1,41.071} = 15.468$, $p = 0.000316$), a borderline-significant effect of trial block (pre-test vs. post-test, $F_{1,93.322} = 3.667$, $p = 0.059$), but no significant main effect of treatment ($F_{1,39.241} = 1.41$, $p = 0.242$). The two-way interaction between treatment and noise amplitude was not significant ($F_{1,41.071} = 0.688$, $p = 0.412$), nor was the interaction between treatment and trial block ($F_{1,93.322} = 0.01$, $p = 0.920$). The three-way interaction between treatment, trial

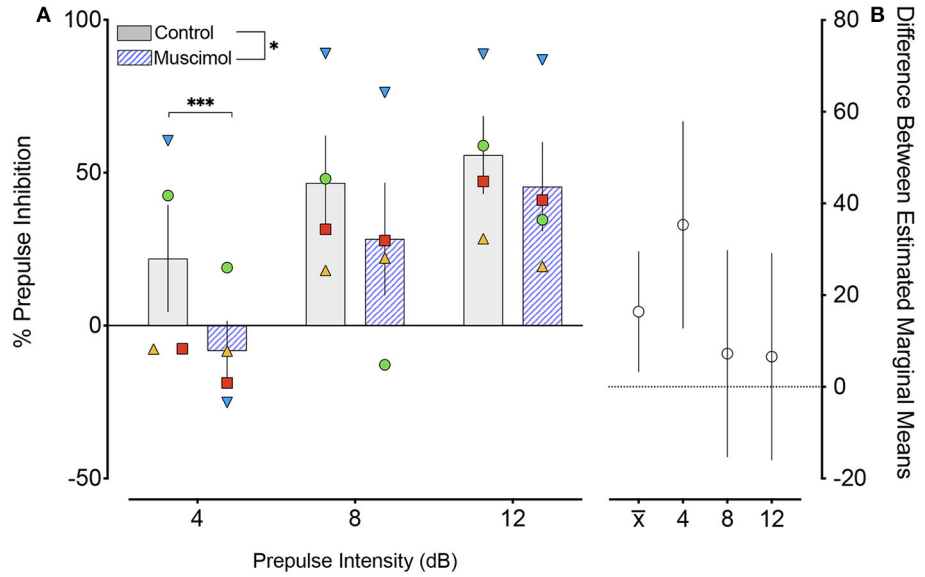

FIGURE 2 | Muscimol infusion into the DLSC impairs PPI. **(A)** % PPI as a function of pre-pulse intensity (dB). Data show a main effect of drug treatment (*$P < 0.05$), apparent in the decreased % PPI following muscimol infusion. Pairwise comparisons revealed a significant decrease only with pre-pulse intensity of 4 dB (***P=0.002). The individual values for each animal are plotted as symbols (SL, blue triangle; OD, red square; RE, yellow triangle; NO, green circle). **(B)** Differences (and 95% confidence intervals) in estimated marginal means for the main effect of treatment ($\bar{x}$) and each pre-pulse intensity.

block, and noise amplitude also did not reach the level of statistical significance ($F_{2,100.296} = 1.150$, $p = 0.321$). Given the trend toward a main effect of trial block, we also examined habituation of startle **Figure 4C**). We found no significant effect of treatment ($F_{1,33.224} = 0.006$, $p = 0.937$), noise amplitude ($F_{2,56.374} = 1.667$, $p = 0.198$), or a treatment-by-noise amplitude interaction ($F_{2,56.676} = 0.575$, $p = 0.566$).

DISCUSSION

Here we report that inactivation of the DLSC by microinjection of the $GABA_A$ agonist, muscimol, significantly disrupts PPI, as measured through a whole-body startle response paradigm, in rhesus macaques without altering startle amplitude. These data add to a small but growing literature examining the pharmacology, physiology, and neural circuitry controlling PPI in non-human primates.

The only other non-human primate study to examine the superior colliculus in the context of PPI—conducted by Saletti et al. in two capuchin monkeys—noted reduced PPI in both lesioned animals (Saletti et al., 2014). Our present findings, as well as the findings from Saletti et al. are consistent with the impairments in PPI observed following SC lesions in rats (Fendt et al., 1994). More generally, our findings also support the well-established role of the DLSC as a sensorimotor integrator. The SC receives visual, auditory, and somatosensory information and influences motor outputs (May, 2006). In rodents, two categories of motor responses are observed upon stimulation of the SC: one class is characterized by orienting responses, such as tracking or pursuit, and the second is characterized by defensive movements, such as avoidance or flight responses (Dean et al., 1989). These behaviors are topographically organized within the SC, with the medial DLSC playing an important role in avoidance behavior and the lateral DLSC playing a role in orienting and approach (Sahibzada et al., 1986; Dean et al., 1989). These fundamental avoidance and approach motor programs are mediated by uncrossed and crossed projections through the brainstem, respectively (Dean et al., 1989). In primates, disinhibition of the DLSC with bicuculline methiodide results in universal defensive responses, regardless of medial vs. lateral activation (DesJardin et al., 2013). While the small number of subjects in the present study precludes a definitive assessment of medial-lateral topography for effects on PPI, we note that both our most medial case (SL) and our most lateral case (NO) showed deficits in PPI after muscimol infusion. A lack of topography in the monkey would be consistent with our prior report on defense-like behaviors (DesJardin et al., 2013). Thus, despite the differences in topography and behavior observed following activation of the rodent SC and the nonhuman primate SC, the role of the SC appears conserved in the context of PPI.

We observed no significant effect of DLSC inactivation on startle habituation during the testing sessions, consistent with a report in rodents showing a lack of effect of superior colliculus lesions on acoustic startle or habituation to startle (Groves et al., 1974). However, this similarity must be interpreted with caution as we did not observe robust startle habituation under baseline conditions. While startle habituation has been routinely reported in mouse and rat studies (Groves et al., 1974; Anisman et al., 2000; Geyer and Swerdlow, 2001; Sandner and Canal, 2007; Partridge et al., 2016), neither we (present study) nor others (Parr et al., 2002) found significant startle habituation in the macaques. This is notable, as our PPI paradigm used a larger number of

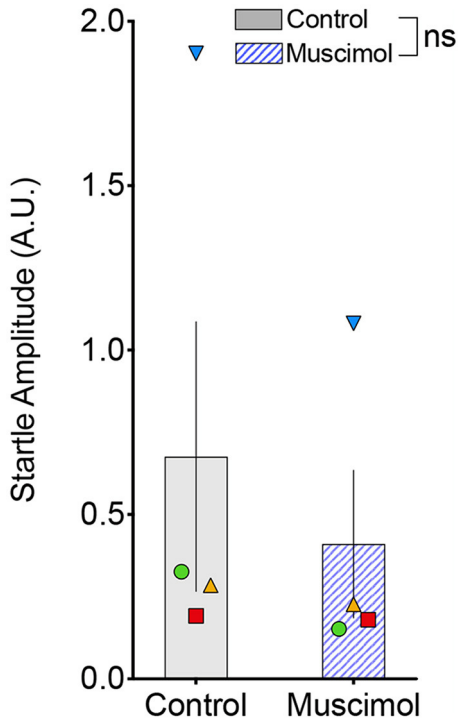

FIGURE 3 | Muscimol infusion into the DLSC does not alter startle amplitude on pulse-alone trials. Each symbol indicates the average startle amplitude for each subject (SL, blue triangle; OD, red square; RE, yellow triangle; NO, green circle) following control or muscimol infusion. Average startle amplitude did not differ between treatment conditions.

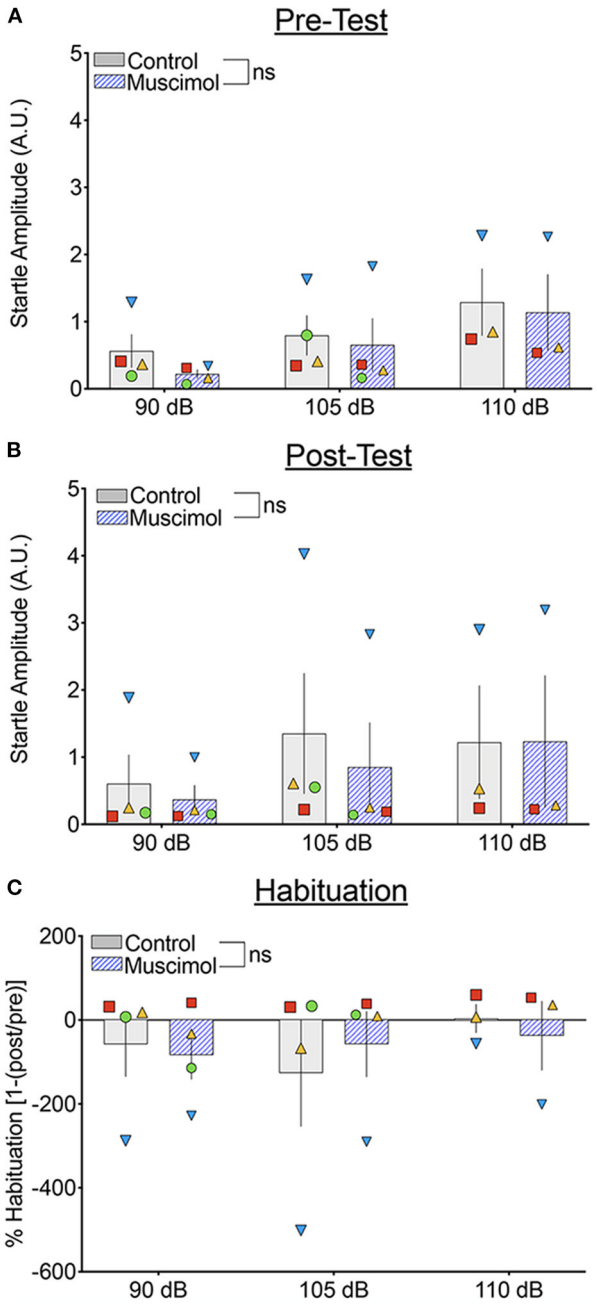

FIGURE 4 | Muscimol infusion into the DLSC does not alter baseline startle amplitude or startle habituation during pre- or post-test trial blocks. Each symbol indicates the average startle amplitude for each subject (SL, blue triangle; OD, red square; RE, yellow triangle; NO, green circle) following control or muscimol infusion. **(A)** Startle amplitude as a function of drug treatment and noise intensity during the pre-test trial block. **(B)** Startle amplitude as a function of drug treatment and noise intensity during the post-test trial block. **(C)** Percent habituation of the startle response comparing the pre and post-test trial blocks.

habituation trials than are typically used in rodents. Similar to our findings, Saletti et al. found no evidence for startle habituation in capuchins. However, habituation to startle-inducing broadband noise has previously been reported in squirrel monkeys (Parker et al., 2011). Moreover, in a fear-potentiated startle paradigm in macaques, within-session habituation was observed between the first trial block and subsequent trial blocks (Winslow et al., 2007), although this effect was modest in the absence of a conditioned stimulus. Finally, Schneider et al. reported significant habituation to startling stimuli across the first four trials of an acoustic startle test in macaques (Schneider et al., 2013). Unfortunately, the remaining studies of acoustic startle (Javitt and Lindsley, 2001; Sánchez et al., 2005; Henry et al., 2009), fear potentiated startle (Antoniadis et al., 2007, 2009; Kazama et al., 2012, 2013), or PPI (Javitt and Lindsley, 2001; Linn and Javitt, 2001; Linn et al., 2003; Nelson et al., 2009; Morris et al., 2010) in non-human primates did not report data regarding startle habituation. Accordingly, there is not a clear consensus on the degree to which habituation of the whole-body ASR is a robust effect in non-human primates.

PPI is disrupted in several neuropsychiatric disorders, including schizophrenia, obsessive compulsive disorder, and PTSD (Grillon et al., 1996; Kohl et al., 2013). Understanding the circuit organization of these disorders critically relies on homology between models and humans, and non-human primates offer both behavioral and circuit homology that makes them ideally suited for this purpose. Particularly in light of our previous findings of species differences in PPI circuitry, we suggest that further evaluation of the neural circuitry controlling

sensorimotor gating in the primate will be informative and of translational value for human neuropsychiatric conditions. Compared to the more extensive literature on acoustic and fear potentiated startle, there have been far fewer studies investigating PPI in primate models as compared to rodents, and fewer still examining circuitry underlying PPI in primates (a total of four studies, including the present). In addition to the study by Saletti et al. described above in capuchin monkeys, the only other circuit-based studies have been from our labs and have focused on the SNpr and the basolateral amygdala. In both cases, findings differed strikingly from those observed in rodents. We found that inactivation of the basolateral amygdala facilitated PPI in macaques (Elorette et al., 2020), a pattern opposite to that observed in rodents, where both permanent damage or transient inhibition of the basolateral amygdala disrupts PPI (Wan and Swerdlow, 1996; Fendt et al., 2000; Forcelli et al., 2012). Similarly, we reported that inactivation of the SNpr in macaques facilitated PPI, whereas in rats it impaired PPI (Aguilar et al., 2018). Following SNpr inactivation, we observed behaviors that we and others have previously shown to be mediated by disinhibition of the SC (DesJardin et al., 2013; Aguilar et al., 2018). We thus hypothesized that this species difference was due to our selective targeting of nigral neurons projecting to the DLSC; these neurons do not collateralize in the primate, but do collateralize and project to other regions (e.g., the pedunculopontine nucleus) in rodents (Yasui et al., 1995; Mailly et al., 2003; Cebrián et al., 2005).

While the effects of inhibition of the DLSC appear consistent across species, we did not study the effect of disinhibition of the DLSC on PPI in the present study. Given that disinhibition of the DLSC can produce motor confounds (defensive responses, postural asymmetries), we were concerned that interpretation of whole-body startle responses would be ambiguous in primates. However, it is well-established that inhibition of the SNpr (as in our prior study) produces a *disinhibitory* (activating) effect on the DLSC. In rodents, intracollicular microinjection of picrotoxin, a GABA chloride channel blocker, enhances PPI without altering ASR amplitude (Fendt, 1999). Similarly, electrical stimulation of the DLSC inhibits the startle reflex in rodents (Li and Yeomans, 2000). Given that our present findings with inhibition of the DLSC are consistent with those reported in rodents—and were expected based on the functional relationship between the SNpr and DLSC—it is likely that nigral projections to the pedunculopontine nucleus in the rodent may explain the previously reported species difference (Aguilar et al., 2018). In rodents, both increased or decreased activity of the PPN results in PPI deficits, suggesting that the PPN is highly sensitive to alterations in neurotransmission in the context of PPI (Koch et al., 1993; Swerdlow and Geyer, 1993; Koch and Schnitzler, 1997; Takahashi et al., 2007). In primates, the role of the PPN in PPI is completely unexplored.

Here, our data suggest that activity within the DLSC is necessary for normal PPI in macaques, as has been reported in rodents. Our findings differ from reports from our labs following inhibition of the SNpr or basolateral amygdala, which showed opposing results in macaques (increased PPI) and rats (decreased PPI). As so little is known regarding the functional organization of PPI circuitry in the primate, both the similarities and the differences highlight the importance of better understanding of these circuits. Given the diverse neuropsychiatric and neurological conditions in humans that are associated with disrupted PPI, a more detailed understanding of the circuitry regulating this phenomenon in the primate brain is clearly warranted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Georgetown University Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

BA, LM, and PF: conceptualization and design. HW, BA, LM, and PF: experimentation, wrote and edited manuscript. HW, BA, and PF: formal analysis. LM and PF: supervised research. All authors contributed to the article and approved the submitted version.

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MacaquePose: A Novel "In the Wild" Macaque Monkey Pose Dataset for Markerless Motion Capture

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Video-based markerless motion capture permits quantification of an animal's pose and motion, with a high spatiotemporal resolution in a naturalistic context, and is a powerful tool for analyzing the relationship between the animal's behaviors and its brain functions. Macaque monkeys are excellent non-human primate models, especially for studying neuroscience. Due to the lack of a dataset allowing training of a deep neural network for the macaque's markerless motion capture in the naturalistic context, it has been challenging to apply this technology for macaques-based studies. In this study, we created MacaquePose, a novel open dataset with manually labeled body part positions (keypoints) for macaques in naturalistic scenes, consisting of >13,000 images. We also validated the application of the dataset by training and evaluating an artificial neural network with the dataset. The results indicated that the keypoint estimation performance of the trained network was close to that of a human-level. The dataset will be instrumental to train/test the neural networks for markerless motion capture of the macaques and developments of the algorithms for the networks, contributing establishment of an innovative platform for behavior analysis for non-human primates for neuroscience and medicine, as well as other fields using macaques as a model organism.

Keywords: non-human primate, deep learning, pose estimation, large-scale dataset, behavior analysis

INTRODUCTION

Behavior analyses are fundamental for understanding brain functions and malfunctions (Datta et al., 2019). Motion capture technologies allow the quantification of animal's pose and motion with a high spatiotemporal resolution enabling the study of the relationship between various brain functions and behaviors (Vargas-Irwin et al., 2008; Nagasaka et al., 2011; Mathis and Mathis, 2020). However, attaching the physical markers for the motion capture is often not practical for animal studies, as the markers themselves disturb/change the subject's behavior (Nakamura et al., 2016; Mathis et al., 2018; Berger et al., 2020). Thanks to recent advances in machine vision using deep learning, the video-based markerless motion capture has been developed to a level permitting practical use (Mathis and Mathis, 2020), in which an artificial neural network predicts the location of body parts in a video without the requirement for physical markers, and enabled successful

behavioral studies in rodents (e.g., Cregg et al., 2020; Dooley et al., 2020; Mathis and Mathis, 2020). Macaque monkeys are an important non-human primate model, particularly in the field of neuroscience (Kalin and Shelton, 2006; Capitanio and Emborg, 2008; Nelson and Winslow, 2008; Watson and Platt, 2012). The robust markerless motion capture using deep learning will allow studying various complex naturalistic behaviors in detail, and permit investigation of relationship between naturalistic behaviors and brain functions (Datta et al., 2019; Mathis and Mathis, 2020). Analyzing naturalistic behavior is crucial in brain-science, since the brain evolved from natural behaviors, and various behaviors, such as complex social behaviors, can be observed only in the natural situations (Datta et al., 2019; Mathis and Mathis, 2020). The deep neural networks usually require manually labeled body parts positions in thousands of pictures to learn prediction of the body parts positions in an arbitrary picture. However, such a large labeled dataset for macaque monkeys in the naturalistic scene has not been developed. The lack of this dataset limits the markerless motion capture technology applications for macaque studies (Bala et al., 2020; Berger et al., 2020).

To overcome this limitation, we created a novel open dataset of the manually labeled body part positions (keypoints) for macaques in naturalistic scenes, consisting of >13,000 pictures. We also validated the usefulness of the dataset by training and evaluating an artificial neural network with the dataset. The results revealed that the keypoint estimation performance of the trained network was close to that of a human level. Our dataset will provide basis for markerless motion capture on the naturalistic behaviors.

MATERIALS AND METHODS

Image Data Collection

A total of 13,083 images of macaque monkeys were obtained from the internet or were captured in zoos or the Primate Research Institute of Kyoto University. Images on the internet were obtained through Google Open Images (https://storage.googleapis.com/openimages/web/index.html) by searching for images with a "macaque" tag. Pictures zoos were acquired from the outside of the breeding areas, with granted permission provided by the zoos. Images in the Primate Research Institute of Kyoto University were taken in the breeding fields without causing any specific interventions to the monkeys. The photo capturing in the institute was approved by the Animal Welfare and Animal Care Committee of the Primate Research Institute of Kyoto University and conducted in accordance with the Guidelines for the Care and Use of Animals of the Primate Research Institute, Kyoto University.

Image Data Annotation

The positions of 17 keypoints (nose and left and right ears, eyes, shoulders, elbows, wrists, hips, knees, and ankles) and instance segmentation for each monkey in each of the pictures were first annotated by non-researchers employed by Baobab Inc. (Chiyoda-ku, Japan). As further expertise was required for high-quality monkey annotation, the keypoint labels were then further refined with eight researchers working with macaques at Kyoto University and the University of Toyama, using a custom-made Python script. The keypoints were labeled according to the following guidelines: (1) The keypoints of the limbs (shoulder, elbow, wrist, hip, knee, and ankle) should be located at the center of the joint rotation. (2) Ear, eye, and nose keypoints should be located at the entrance of the ear canal, the center of eye ball, in the middle position between the entrances of the two nostrils, respectively. (3) A keypoint was annotated, if its position was predictable despite being occluded, except for ears, eyes, and nose facing the back side of the picture. The resultant labels were compatible with the Microsoft COCO Keypoint Dataset (Lin et al., 2014).

Performance Evaluation of an Artificial Neural Network Trained With the Present Dataset

To validate the present dataset, we trained an artificial neural network estimating keypoint positions by using the DeepLabCut algorithm proposed for markerless pose estimation in animals (Mathis et al., 2018). Briefly, DeepLabCut is a versatile and straightforward algorithm in which the 50-layer ResNet pre-trained for the ImageNet object recognition task (He et al., 2016) is transferred for the keypoint estimation by replacing the classification layer at the output of the ResNet with the deconvolutional layers (see **Supplementary Figure 1** for the network architecture of the DeepLabCut). The utilization of transfer learning allows DeepLabCut algorithm to require a relatively small number of training data (Nath et al., 2019). The accuracy of keypoint prediction with the DeepLabCut algorithm has been shown to be comparable or superior to similar algorithms recently suggested for the animal pose estimation (Graving et al., 2019). DeepLabCut is a widely used algorithm in the field of neuroscience, because of its user-friendly interface and documentations, and a well-established community, as well as its good performance. Due to DeepLabCut (version 2.1.6) currently not supporting the estimation of keypoints in multiple animals in a picture, we first generated single monkey images by masking the monkeys in the images except for one monkey and used these masked images as the input. Some monkey images in the dataset were excluded due to technical reasons (e.g., a keypoint of one monkey is covered by the mask of the other monkeys). Then, the images were resized to adjust the length to 640 pixels while maintaining the images aspect ratio, before inputting it into the network. In total, 15,476 single monkey images were generated. Among the images, 14,697 single monkey images were used to train the network and the rest (779 images) were used to evaluate the trained network. The network model was implemented using Python scripts with Tensorflow support. The network is trained up to a million iterations. The training took 20 h to complete on a Nvidia GTX 1080 Ti graphics processing unit workstation.

The keypoint prediction by the trained network was evaluated. A predicted keypoint with confidence level > 0.4 was defined to be detected. First, minor cases showing the keypoint(s) detected outside the monkey segment were eliminated. True positive,

FIGURE 1 | Examples of pictures and labels in the present dataset.

true negative, false positive, and false negative detections were counted. A keypoint was defined as a correct detection by the network (true positive detection) if there was the corresponding ground truth keypoint in the same image, regardless of its location in the image. For true positive cases, the Euclidean distance between the predicted and ground truth position was calculated as the error of position estimation. The error value represented the normalized value with respect to the length of the monkey's bounding box due to variations in the size of the monkey in the images. To check the accuracy of the predicted pose, the root-mean-square error (RMSE) was also calculated with all keypoints in each image (Mathis et al., 2018). To evaluate the error values of the keypoint position predictions, we investigated human variability by calculating the errors between the keypoint positions annotated by two humans. Finally, among the true positive cases, numbers of limb keypoints misattributed as the homologous keypoint on another limb (e.g., left wrist misattributed as right wrist, left ankle, or right ankle) are also counted. Specifically, *i*-th keypoint were defined as being misattributed to a homologous *j*-th keypoint on another limb, if the keypoint satisfies both of the following two conditions: (1) the normalized position error of the *i*-th keypoint was >20%; (2) the ground truth positions of *j*-th keypoint was closest to the predicted position of *i*-th keypoint among the ground truth positions of homologous keypoints. Note that these keypoint predictions obtained with the trained network were evaluated on set of test images which are not included during training of the network.

RESULTS

In total, the present data set contains keypoints and instance segmentation of 16,393 monkeys in 13,083 pictures. Each picture captures 1–5 monkeys; 10,630 pictures with a single monkey and 2,453 pictures with multiple monkeys (**Figure 1**).

To validate the dataset, we trained an artificial network with 14,697 single monkey images in the dataset using the DeepLabCut algorithm (Mathis et al., 2018). The performance of the keypoint prediction of the trained network was evaluated on 779 test images unseen during training. **Figure 2** shows examples of the keypoint predictions (see **Supplementary Video 1** for keypoint prediction for movies). Among 779 images, 24 images had keypoint(s) detected outside the target monkey. Most of them (17 images) were due to imperfect masks of the other monkeys in the picture (**Supplementary Figure 2**). The "out of monkey" cases were removed from the analysis.

We investigated the performance of keypoint detection (judging whether a keypoint exists anywhere in the picture or not) of the trained network (**Supplementary Table 1**). Both precision and recall of the keypoint detection were approximately 90% in most of the keypoints, suggesting good detection performance.

FIGURE 2 | Examples of test image predictions. Test images (left), the ground truth keypoint positions (center) and the position predicted by the artificial neural network trained with the present dataset using the DeepLabCut algorithm (right; **a–h**). The inset (top right corner) shows color codes of the keypoints. Red arrows in **(h)** indicate a misattribution error.

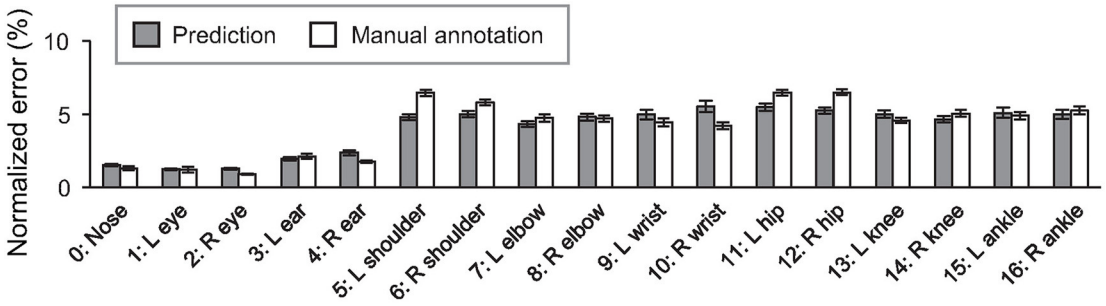

FIGURE 3 | Averaged error of predicted (gray) and manual labeled (white) positions of each keypoint comparing with the ground truth positions. Error bars represent standard error of the mean (s.e.m).

To further investigate the accuracy of the detected keypoints, the error of predicted position was calculated for each keypoint (**Figure 3**, gray bar). The prediction's RMSE values (6.02 ± 0.18%; mean ± s.e.m) were comparable to those between the positions manually labeled by two different people (5.74 ± 0.16%; $p = 0.250$, student's *t*-test), suggesting that the trained network's performance in the keypoint position estimation was close to the human level. The effect of the label refinement by researchers was also examined. The error values for the dataset before the refinement were calculated as previously mentioned. The analyses revealed that the averaged RMSE values after the refinement (6.02 ± 0.18%) were significantly smaller than the one before the refinement (7.83 ± 0.23%; $p = 9.13 \times 10^{-10}$, Student's *t*-test; see **Supplementary Figure 3** for the error value of each keypoint). The result suggests that the network trained with the dataset refined by the researchers predicted the keypoint more consistently.

In some cases, we observed that the predicted positions of monkey's keypoints on a limb were located on homologous keypoints on another limb (**Figure 2h**, see also **Supplementary Video 1**).

We then quantified the frequency of such misattribution errors (**Table 2**). The misattribution errors were relatively frequent in the distal keypoints (elbow, knee, wrist, and ankle), especially on the hind limbs. The total number of images having at least one misattribution error was 114 (15%). The result shows that there is still room for improvement, although the RMSE indicates human-level performance.

DISCUSSIONS

In this study, we created a novel large dataset of labeled keypoints of macaque monkeys (**Figure 1**, **Table 1**). The keypoint estimation performance of the neural network trained with the dataset was close to that of human level (**Figures 2**, **3**; **Supplementary Video 1**), demonstrating the usefulness of the present dataset. We also found a significant improvement of the network prediction after the label refinement by researchers using macaques (**Supplementary Figure 3**), suggesting that the refinement successfully enhanced the quality of the dataset. Although we tested only single monkey images due to the limitation of the algorithm, the present dataset should be useful to train/test the network for multi-animal motion capture. The label formats in the present dataset are compatible with those used in the COCO dataset for humans (Lin et al., 2014), allowing users to try a direct application of algorithms developed for human motion capture. A recent study also proposed a similarly sized labeled dataset of rhesus monkeys (Bala et al., 2020). In the study, they captured freely moving monkeys in a 2.5 m cubic cage with 62 cameras surrounding the cage. The multi-camera system allows to reconstruct 3D pose after manually labeling images simultaneously captured from 3 to 4 views. Interestingly, the reconstructed 3D pose is projected to the other around 60 views and enables automatically labeling the images from all the views. This cross-view data augmentation allowed them to get labels of around 200,000 monkey images with 33,192 images labeled manually. The critical difference between the two datasets is that pictures in their dataset were taken in a single laboratory environment, our dataset consists of pictures taken in many different naturalistic environments. Thanks to the "in-the-wild" aspect of the collected pictures, the present data set has rich variations in pose, body shape, lighting, and background in naturalistic contexts. The rich variation will help to train and test artificial neural networks with high generalizability (Mathis et al., 2019). Thus, the two datasets will compensate each other to train or test better neural networks in future studies. As the dataset formats (i.e., which keypoints are labeled) were slightly different among the two datasets, some additional efforts are necessary to combine or compare these two datasets directly.

To understand how the brain generates our behavior, analyzing naturalistic behaviors is crucial. The brain evolved from natural behaviors, and various behaviors, such as complex social behaviors, can be observed only in the natural situations (Datta et al., 2019; Mathis and Mathis, 2020). The high-resolution spatiotemporal data obtained with the markerless motion capture will also aid in understanding brain dynamics underlying the behavior (Berger et al., 2020). Specific posture and motion are informative for studying animals' emotions and intension (Nakamura et al., 2016), and the motor functions (Berger et al., 2020). Furthermore, the automatic and long-term analyses of naturalistic behavior from a large number of subjects permit new data-driven approaches to find unusual behaviors, personalities and, underlying genetic and neural mechanisms (Vogelstein et al., 2014; De Chaumont et al., 2019). For instance, the recently discovered autistic traits exhibited by macaque monkeys (Yoshida et al., 2016) was identified by such a behavioral

TABLE 1 | The number of pictures and monkeys in the present dataset from each source.

Source	Monkey Species	No. of Pictures	No. of Monkeys
Toyama Municipal Family Park Zoo	Japanese Macaque	3,784	4,952
Itozu no Mori Zoological Park	Japanese Macaque	1,312	1,622
Primate Research Institute	Japanese Macaque	1,641	2,131
Inokashira Park Zoo	Rhesus Macaque	2,747	3,203
Tobu Zoo	Rhesus Macaque	2,461	2,755
Google Open Images	Various	1,138	1,730
Total		1,3083	1,6393

TABLE 2 | Number of the misattribution errors.

Keypoint pairs	Correct	L-R incorrect	F-H incorrect	L-R and F-H incorrect	Total
Shoulder	1,225	5	1	3	9
Hip	1,062	7	2	3	12
Elbow	1,134	11	5	4	20
Knee	1,066	45	11	3	59
Wrist	1,049	14	7	6	27
Ankle	1,045	29	11	5	45

L-R incorrect referring to left or right predicted keypoint was incorrect; F-H switch, forelimb or hindlimb label was incorrect.

observation. Thus, the markerless motion capture for macaque monkeys developed based on the present dataset will be of great use for many neuroscience studies.

The performance evaluation of the network trained with the present dataset revealed that there is still room for improvement regarding the misattribution of the limb keypoints (**Figure 2h**, **Table 2**), although the RMSE indicates the human-level performance (**Figure 3**). The DeepLabCut algorithm (Mathis et al., 2018) used in the present evaluation does not explicitly utilize the prior knowledge about the animal's body, whereas the other algorithms were suggested to use the connection between keypoints (Insafutdinov et al., 2016; Cao et al., 2017) or 3D shape of the subject (Biggs et al., 2018; Zuffi et al., 2019). Such utilization of the prior knowledge may help to improve the estimation. However, even the state-of-the-art human motion capture algorithms also have difficulties in analyzing the pictures with severe occlusion or crowded people (Mathis and Mathis, 2020). Due to severe occlusions more frequently being observed in naturalistic behaviors in monkeys than in humans, better algorithms may be required in the future. An alternative approach for the improvement will be enriching the dataset itself. Although we tried to capture many different poses in various contexts, the sampling was biased to the frequently observed poses. Adding data selectively for the rarely observed poses may improve the performance of the trained network. Combining with the other monkey datasets made for laboratory environments (Bala et al., 2020; Berger et al., 2020) or transfer learning of the network trained with the human dataset (Sanakoyeu et al., 2020) are also interesting approaches. Nevertheless, in practice, the performance of the network shown in the present study may be sufficient for many applications, after appropriate temporal filtering of the motion data (Berman et al., 2014; Nath et al., 2019) and additional training with the labels made on the pictures in the target experiment (Mathis et al., 2019).

In the present study, we evaluated the keypoint estimation in 2D images by the neural network. However, for the next step of behavior analysis, the researchers would need to reconstruct the 3D pose and motion of the animals (Nath et al., 2019; Bala et al., 2020) then label the behaviors that the animals are exhibiting based on the estimated pose and motion (Datta et al., 2019). The post-processing methods for converting the high-dimensional motion data into meaningful and interpretable behavioral events and parameters of a single animal or interacting animals are still under active developments (Berman et al., 2014; Datta et al., 2019; Dviwedi et al., 2020). The present dataset will permit simple access to motion data of macaques in various environments, and this could accelerate the development of post-processing method by accumulating the motion data associated with various natural behaviors. It is also interesting to add labels of monkey behavior (e.g., running, eating, sleeping, grooming, fighting, etc.) engaged in each picture in the present dataset, for the development of the behavioral event detection methods.

CONCLUSION

We created a novel large open dataset of keypoint labels of macaques in naturalistic scenes. The dataset will be instrumental to train/test the neural networks for markerless motion capture of the macaques and developments of the algorithms for the networks, contributing to the establishment of an innovative platform of behavior analysis for non-human primates for neuroscience and medicine, as well as the other fields using macaques (Carlsson et al., 2004).

DATA AVAILABILITY STATEMENT

The dataset for this study is publicly available on the website of Primate Research Institute, Kyoto University (http://www.pri.kyoto-u.ac.jp/datasets/). The trained network model described in the present paper is readily available through DeepLabCut Model Zoo (http://www.mousemotorlab.org/dlc-modelzoo). The other raw data supporting the conclusions of this article will be made available by the authors upon request.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Welfare and Animal Care Committee of the Primate Research Institute of Kyoto University.

AUTHOR CONTRIBUTIONS

RL, TS, JM, KI, YG, HNishij, and HNishim designed this research. JM, KI, TS, RL, and MT created the dataset. RL, SBN, JM, and TS evaluated the performance of the neural network trained with the dataset. All the authors discussed the results and commented on the manuscript, read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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Preferential Neuronal Responses to Snakes in the Monkey Medial Prefrontal Cortex Support an Evolutionary Origin for Ophidiophobia

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Ophidiophobia (snake phobia) is one of the most common specific phobias. It has been proposed that specific phobia may have an evolutionary origin, and that attentional bias to specific items may promote the onset of phobia. Noninvasive imaging studies of patients with specific phobia reported that the medial prefrontal cortex (mPFC), especially the rostral part of the anterior cingulate cortex (rACC), and amygdala are activated during the presentation of phobogenic stimuli. We propose that the mPFC-amygdala circuit may be involved in the pathogenesis of phobia. The mPFC receives inputs from the phylogenically old subcortical visual pathway including the superior colliculus, pulvinar, and amygdala, while mPFC neurons are highly sensitive to snakes that are the first modern predator of primates, and discriminate snakes with striking postures from those with non-striking postures. Furthermore, the mPFC has been implicated in the attentional allocation and promotes amygdala-dependent aversive conditioning. These findings suggest that the rACC focuses attention on snakes, and promotes aversive conditioning to snakes, which may lead to anxiety and ophidiophobia.

Keywords: snakes, phobia, rostral anterior cingulate cortex, amygdala, snake detection theory

INTRODUCTION

Specific phobia is a type of anxiety disorder with a lifetime prevalence ranging from 3% to 15% across various countries (Eaton et al., 2018). Patients with specific phobia display immediate excessive fear or anxiety in response to a specific object or situation despite the absence of actual danger imposed by the specific object or situation, and the disturbances by specific phobia impose a significant impact on patients' daily activity

(American Psychiatric Association, 2013). Specific phobia consists of five types, and ophidiophobia (snake phobia) belongs to an animal type of specific phobia. Psychological studies reported that 53.3% of survey participants felt anxiety in response to snakes (Davey, 1994), and 2–3% of the participants were ophidiophobia-like (Klorman et al., 1974; Klieger, 1987; Polák et al., 2016), and that ophidiophobia is one of the most common specific phobias (Fredrikson et al., 1996).

It has been proposed that this specific phobia may have an evolutionary origin (Mineka and Öhman, 2002; Russell et al., 2015; Rakison, 2018). The fear module of primates, including the superior colliculus, pulvinar, and amygdala, may have evolved particularly in response to the threat posed by snakes (Isbell, 1994, 2006, 2009; Öhman and Mineka, 2003; Soares et al., 2017). Consistently, monkey pulvinar neuronal activity is more sensitive to snake images than other control images (Le et al., 2013, 2014, 2016). Furthermore, human and monkey behavioral studies support the existence of an innate fear module sensitive to snake images. Human infants (5–6 months old) who likely had less experience of snakes looked at snake images longer than control images and displayed stronger sympathetic responses to snake images (Hoehl et al., 2017; Rakison, 2018), while young children and adults detected snakes faster than control images (LoBue and DeLoache, 2008; Masataka et al., 2010). In monkeys, isolation-reared (snake-naïve) mouse lemurs and pig-tailed macaques avoided a snake model and odor of snakes (Nelson et al., 2003; Weiss et al., 2015). Also, humans are predisposed to efficiently learn aversive conditioning between snakes and unconditioned shock (Seligman, 1970; Öhman and Mineka, 2003). These findings suggest that the innate fear module for snake detection and learning may be involved in snake phobia.

It has been proposed that attentional bias to specific animals may promote anxiety (i.e., phobia; Matthews and MacLeod, 2002; Heeren et al., 2013; LoBue and Rakison, 2013): images of specific animals are automatically processed in a fear module regardless of attention (Öhman and Soares, 1994), compete with cognitive activity, and when the activity for the specific animals exceeds cognitive activity, capture attention for awareness to induce anxiety. Consistent with this hypothesis, snake images, which are prone to induce phobia (see above), captured more attention than other control images in monkeys and humans (Shibasaki and Kawai, 2009; Soares et al., 2014; Kawai and Koda, 2016; Gomes et al., 2017). It is suggested that the anterior cingulate cortex (ACC) is involved in attentional allocation to emotional stimuli (Niedenthal and Kitayama, 1994) and that the affective (rostral) part of the ACC (rACC) is involved in salience evaluation of emotional stimuli (Bush et al., 2000). Furthermore, phobogenic stimuli were detected faster than control stimuli in subjects with strong fear comparable to specific phobia and patients with phobia (Öhman et al., 2001; Bar-Haim et al., 2007), while the rACC was activated in response to phobogenic stimuli including snakes in patients with specific phobia (Rauch et al., 1995; Pissiota et al., 2003; Carlsson et al., 2004; Amir et al., 2005; Straube et al., 2007; Britton et al., 2009). Thus, the currently available data suggest that the ACC, especially rACC, plays an important role in the pathogenesis of ophidiophobia.

Based on evolutional theories for the origin of phobias (see above), we hypothesized that the ACC, which receives projections from the pulvinar (Porrino et al., 1981; Romanski et al., 1997), is also sensitive to snakes. Consistently, neurons in the medial prefrontal cortex (mPFC) including the ACC responded faster and stronger to snakes in monkeys (Dinh et al., 2018). Furthermore, behavioral studies reported that monkeys, as well as humans, are more sensitive to striking postures in snake detection and threat assessment (Masataka et al., 2010; Etting et al., 2014). Based on these behavioral findings and evolutionary reasoning, here we tested whether mPFC neurons are more responsive to images of snakes with striking postures in non-human primates.

THE BEHAVIORAL TASK FOR NEUROPHYSIOLOGICAL RECORDING FROM THE MONKEY mPFC

To test this prediction, we recorded and analyzed monkey mPFC neuronal responses to snake images with striking and non-striking postures. The recording was performed using two adult macaque monkeys (*Macaca fuscata*: 1 female and 1 male) weighing 7.1–8.6 kg, which was the same monkeys used in the previous experiment (Dinh et al., 2018). In the previous study (Dinh et al., 2018), the responses of mPFC neurons to snake images regardless of their postures were compared with other categories of images (e.g., raptors, carnivores, humans, and monkey faces, et cetera). In this study, the neuronal responses to snake images with striking and non-striking postures were analyzed. While the monkeys were individually housed with food available *ad libitum*, the monkeys' water intake was restricted in their home cages and they obtained juice rewards during training as well as recording experiments (Dinh et al., 2018). The monkeys were treated according to the United States Public Health Service Policy on Human Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Guidelines for the Care and Use of Animals at the University of Toyama. Experimental procedures in this study have been approved by the Ethical Committee for Animal Experiments at the University of Toyama (Dinh et al., 2018).

The detailed procedures are described in Dinh et al. (2018). Briefly, the monkey sat in a monkey chair in front of a computer display. Eye-movements were monitored by a CCD camera (Matsuda, 1996). In the present study, six snake photos consisting of three snake images with striking postures and another three snake images with non-striking postures were used (**Figure 1**). The present study used the same visual stimuli in the previous study in the monkey pulvinar (Le et al., 2014). The monkeys discriminated against the snake images in a sequential delayed nonmatching-to-sample task (DNMS; Dinh et al., 2018). Briefly, the task started with a buzzer tone, followed by a fixation cross for 1.5 s. Then, a sample stimulus was presented for 500 ms (sample phase) between 1 and 4 times with a 1.5-s interval. After a 1.5-s interval from the last presentation of the sample stimulus, a new stimulus (target phase) was presented. The monkey could

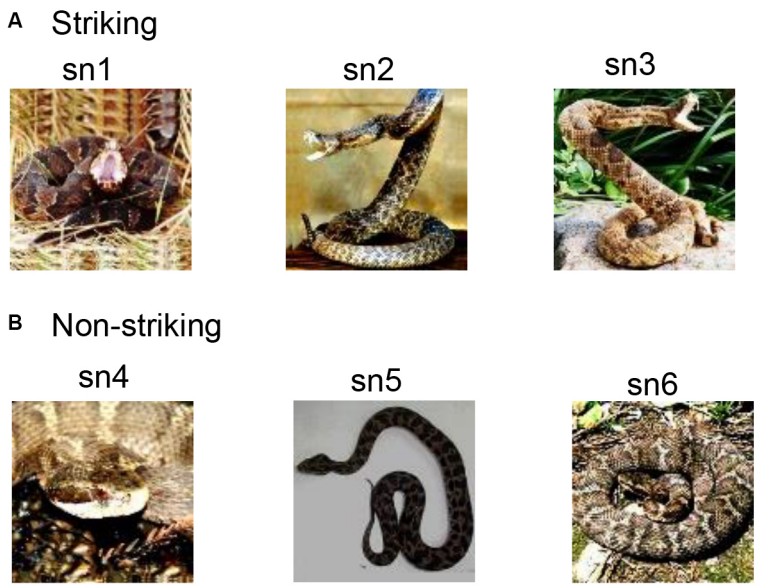

FIGURE 1 | Visual stimuli used in a neurophysiological recording from the monkey medial prefrontal cortex (mPFC). **(A)** Three photos of snakes with striking postures. **(B)** Three photos of snakes with non-striking postures. The same visual stimuli used in the study by Le et al. (2014) were also used in the present study.

obtain a juice reward if the monkey pressed a button in the chair within 2 s.

Upon reaching a 96% correct-response criterion in the DNMS task, a U-shaped epoxy-resin plate was attached to the skull under anesthesia (Nishijo et al., 1988; Tazumi et al., 2010; Dinh et al., 2018). The monkeys were retrained with the DNMS task two weeks after the surgery, while the head was painlessly fixed using a head-restraining device for the U-shaped plate. After re-training of the monkeys with the head fixed, neuronal activity was recorded from the mPFC during the DNMS task. The detailed procedures for electrophysiological recording were reported previously (Dinh et al., 2018). Briefly, a glass-coated tungsten microelectrode was stereotaxically inserted into the mPFC to record neuronal activity. All the data, including analog signals of neuronal activities, were stored in a computer off-line analysis.

CHARACTERISTICS OF mPFC NEURONAL RESPONSES TO SNAKE POSTURES

Only neuronal responses to sample stimuli were analyzed (Dinh et al., 2018). Briefly, significant responses to each snake image were determined by a Wilcoxon signed–rank test ($p < 0.05$), in which neuronal activity during 500-ms after (post) stimulus onset was compared with that during 100-ms before (pre) stimulus onset. The mPFC neurons were defined as snake-responsive neurons if a given mPFC neuron showed significant responses to at least one of the six snake images. The 235 mPFC neurons were tested with all six snake images in the DNMS task, and 95 neurons responded to one or more snake images (snake-responsive neurons). The numbers of mPFC neurons and snake-responsive neurons in the individual monkeys are indicated in **Supplementary Table 1**. There were no significant differences in the proportion of snake-responsive neurons among the mPFC neurons between the two monkeys (χ^2-test; $\chi^2_{(1)} = 1.147$, $p = 0.284$). **Figure 2** shows a representative snake-responsive mPFC neuron. The neuron responded stronger to snakes with striking postures (**Figure 2A**). For each snake-responsive neuron, response magnitudes to each stimulus were computed [i.e., (mean firing rates during the post 500 ms period) minus (mean firing rates in the pre 100 ms period)]. There was a significant difference in the response magnitudes among the six snake images in this neuron (one-way ANOVA: $F_{(5,63)} = 6.215$, $p < 0.0001$; **Figure 2B**). Subsequent *post hoc* tests indicated that response magnitudes to the three snake images with striking postures were larger than those to the three snake images with non-striking postures (Tukey test, $p < 0.05$).

Snake-responsive neurons were further categorized based on comparison of response magnitudes to the snake images with striking postures with those to non-striking postures. For this categorization, two peri-event histograms, one for the snakes with striking postures and the other for the snakes with non-striking postures, were constructed. The mPFC neurons were categorized as striking-selective neurons if the response magnitudes to the three snake images with striking postures were larger than those to the three snake images with non-striking postures (unpaired *t*-test, $p < 0.05$), while the mPFC neurons were categorized as non-striking-selective neurons if the response magnitudes to the three snake images with non-striking postures were larger than those to the three snake images with striking postures (unpaired *t*-test, $p < 0.05$). The remaining mPFC neurons were defined as nonselective neurons. Of the 95 snake-responsive mPFC

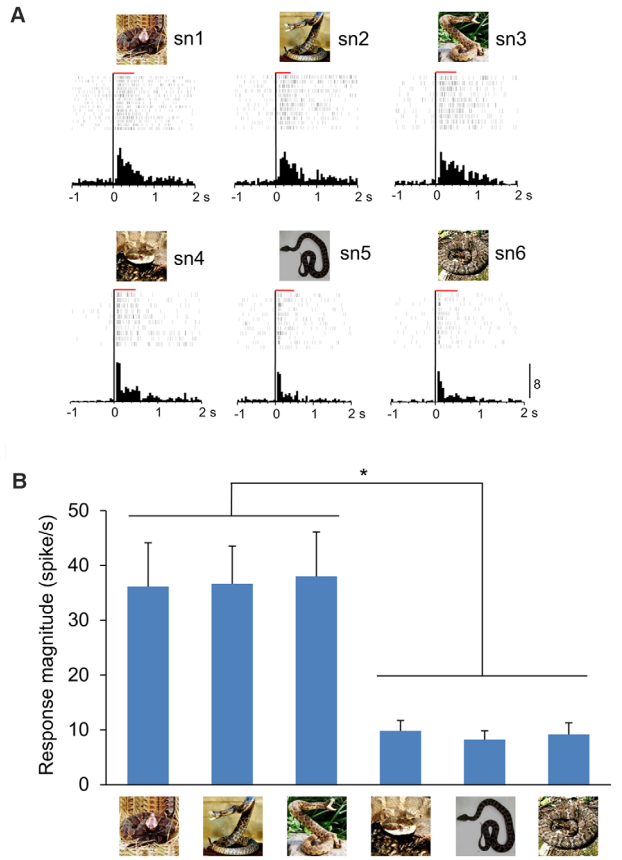

FIGURE 2 | A representative mPFC neuron sensitive to snake postures. **(A)** Peri-event histograms of the mPFC neuronal activity in response to each snake photo. Each raster display above each histogram indicates neuronal activity. The red horizontal bars above the raster display indicate the stimulus presentation period (500 ms). Zero in the abscissas indicates the stimulus onset. Calibration at the right bottom of the figure indicates the number of spikes per trial in each bin. Bin width = 50 ms. **(B)** Response magnitudes of this neuron to the six snake images. Histograms indicate mean ± SEM. *$p < 0.05$.

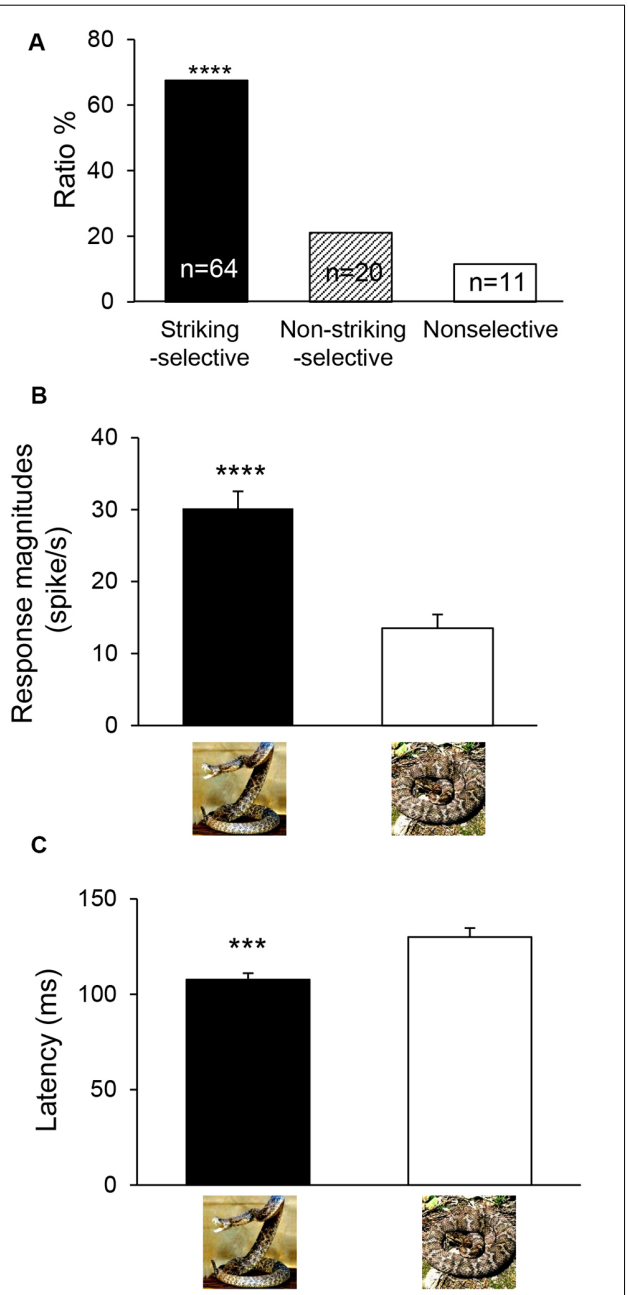

FIGURE 3 | Classification **(A)** and response characteristics **(B,C)** of snake-responsive neurons. **(A)** The ratios of three types of mPFC neurons to the snake postures. ****Significant difference from non-striking-selective mPFC neurons ($p < 0.0001$). **(B)** Comparison of mean response magnitudes to snakes with striking postures and those to non-striking postures ($n = 95$). ****Significant difference from snakes with non-striking postures ($p < 0.0001$). **(C)** Comparison of mean response latencies to snakes with striking postures and those to non-striking postures ($n = 87$). ***Significant difference from snakes with non-striking postures ($p < 0.001$).

neurons, 64 neurons responded stronger to snakes with striking postures (striking-selective), 20 neurons responded stronger to snakes with non-striking postures (non-striking-selective), and 11 neurons showed no difference in response magnitudes between these two categories of the snake images (non-selective; **Figure 3A**). Since there were no significant differences in the proportion of striking-selective neurons among the mPFC neurons between the two monkeys (χ^2-test; $\chi^2_{(1)} = 0.095$, $p = 0.758$), the combined data from the two monkeys were further analyzed. The ratio of the striking-selective neurons was significantly greater than that of the non-striking-selective neurons (χ^2-test; $\chi^2_{(1)} = 20.51$, $p < 0.0001$). Furthermore, averaged response magnitudes of all snake-responsive neurons to the snakes with striking postures and those to the snakes with non-striking postures were compared by paired t-tests ($p < 0.05$). The results indicated that the averaged response magnitudes to the snakes with striking postures were significantly larger than those to the snakes with non-striking postures (paired t-test: $t_{(94)} = 5.459$, $p < 0.0001$; **Figure 3B**).

Response latencies to the snake images were also analyzed. For each neuron, the same two data sets used to analyze

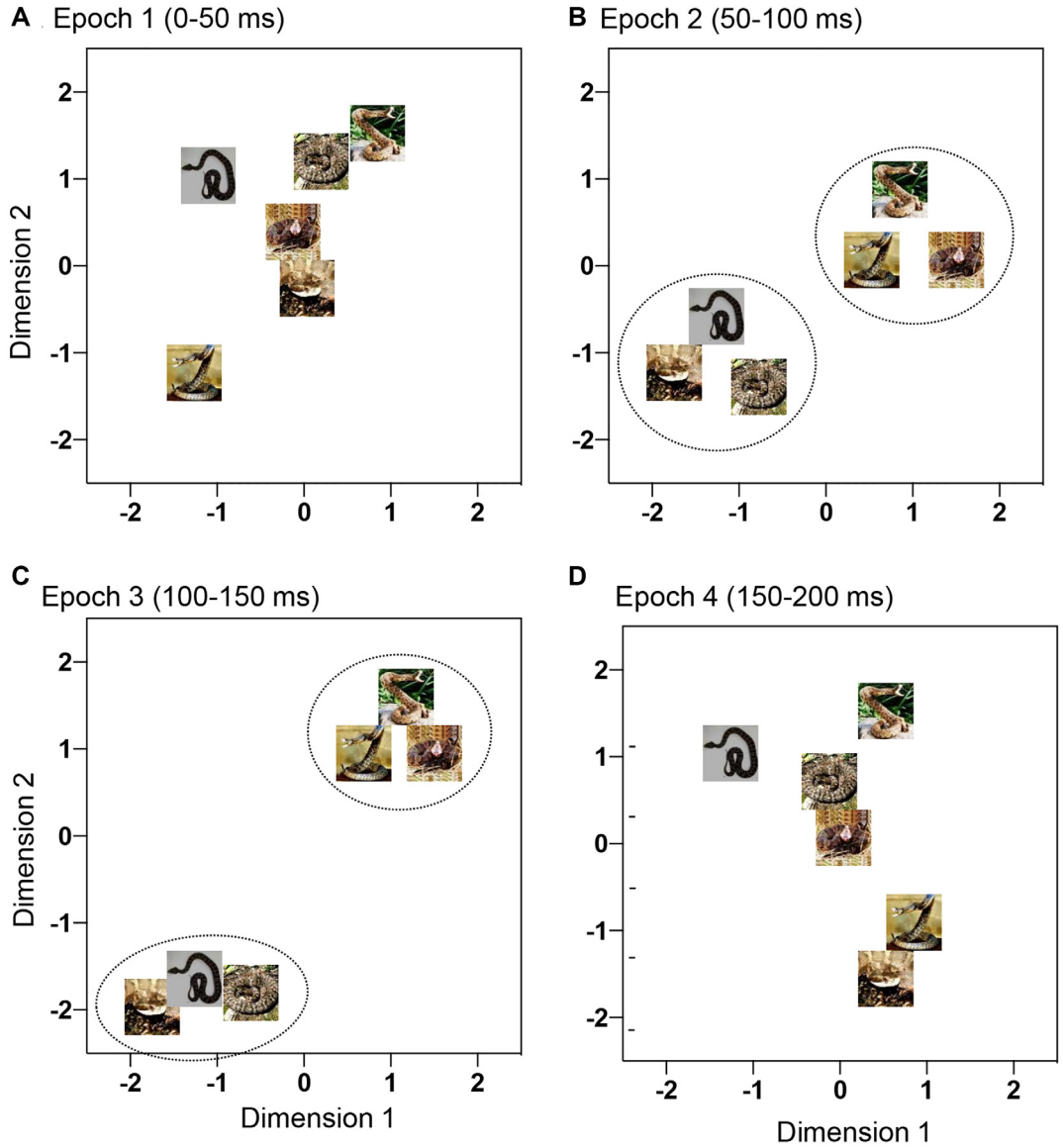

FIGURE 4 | Distributions of the six snake photos in a 2-D space derived from multidimensional scaling (MDS) analysis of response magnitudes of the 95 mPFC neurons to these photos. **(A–D)** Response magnitudes were analyzed by MDS epoch 1 **(A)**, epoch 2 **(B)**, epoch 3 **(C)**, and epoch 4 **(D)**. In epochs 2 and 3 **(B,C)**, the snakes with striking postures were separated from the snakes with non-striking postures ($p < 0.05$).

response magnitudes for snakes with striking and non-striking postures (see above) were also used to analyze response latencies. Response latency of the mPFC neuron was measured as the period between the stimulus onset and the time point at which the neuronal firing rate exceeded the mean ± 2 SD of the baseline firing rate. The mean response latencies of all striking-selective neurons were compared to non-striking-selective neurons by paired *t*-tests ($p < 0.05$; $n = 87$ for latency measures). The results indicated that averaged response latencies to the snakes with striking postures were significantly shorter than those to the snakes with non-striking postures (paired *t*-test: $t_{(86)} = 3.804$, $p = 0.0003$; **Figure 3C**).

POPULATION CODING OF SNAKE POSTURES IN THE MONKEY mPFC

To investigate temporal changes in population coding of snake postures in the mPFC, the initial 200-ms after stimulus onset was divided into four 50-ms epochs: epoch 1 (0–50 ms), epoch 2 (50–100 ms), epoch 3 (100–150 ms) and epoch 4 (150–200 ms). The mean response magnitude in each epoch was similarly calculated, as follows: (mean firing rate in each epoch) minus (mean firing rate during the 100-ms pre-period). These four data sets were separately analyzed by multidimensional scaling (MDS). MDS can convert the relationships (Euclidean

distances as dissimilarity in this study) between all possible pairs of stimuli to a geometric representation of the stimuli in a space (see Young and Hamer, 1987 for more details). In this study, data matrices of response magnitudes in a 95 × 6 array (95 snake-responsive neurons × 6 stimuli) were generated in each epoch and were subjected to MDS analysis. Then, six visual stimuli were positioned in the two-dimensional space so that the geometrical relationships among the stimuli represented the original relationships (MDS, PROXSCAL procedure, SPSS statistical package, version 16; Shepard, 1962; Kruskal, 1964).

In the 2-D MDS spaces, r^2 values of epochs 1, 2, 3 and 4 were 0.671, 0.819, 0.953, and 0.762, respectively. The distributions of the six snake images in 2D space derived from each epoch data are shown in **Figure 4**. In epoch 2 and 3, two clusters, one for snakes with striking postures and another for snakes with non-striking postures, were recognized. Discriminant analyses of the MDS data indicated that the correct percent of discrimination between the two categories of the snake images was 97.32% and 100% in epochs 2 and 3, respectively ($p = 0.016$ and $p = 0.012$, respectively; **Table 1**). In epochs 1 and 4, the two categories of the snake images were not significantly separated.

A previous study reported that response magnitudes and latencies in the mPFC were positively correlated with the corresponding data in the pulvinar (Dinh et al., 2018). Furthermore, in the previous study in which responses to the same snake images with striking and non-striking postures were analyzed in the pulvinar, snakes with striking postures were significantly separated from those with non-striking postures in epoch 2 in the MDS analysis (Le et al., 2014). This study investigated how the MDS data for the snakes with striking and non-striking postures in the mPFC (this study) were related to those in the pulvinar (Le et al., 2014). To investigate the relationships between the two MDS results, we analyzed the relationships between the Euclidean distances between all stimulus pairs in MDS spaces in epochs 2 and 3 in the mPFC and those in the pulvinar data in epoch 2 (Le et al., 2014) by linear regression analysis. The results indicated that the distances between all possible stimulus pairs in the mPFC MDS in epoch 2 were significantly and positively correlated with the corresponding data in the pulvinar MDS space in epoch 2 ($r^2 = 0.717$; $F_{(1,13)} = 32.97$, $p < 0.0001$), and that the distances in the mPFC MDS in epoch 3 were also significantly and positively correlated with the corresponding data in the pulvinar MDS space in epoch 2 ($r^2 = 0.754$; $F_{(1,13)} = 39.74$, $p < 0.0001$). These findings suggest that the mPFC receives information from the pulvinar to discriminate snake postures.

LOCATIONS OF THE mPFC NEURONS

The histological procedures to identify recording sites in the mPFC were reported previously (Dinh et al., 2018). Briefly, after the last recording session, the monkeys were perfused under deep anesthesia, and brain sections were processed with Cresyl violet. Then, recording sites of mPFC neurons were stereotaxically determined based on the stereotaxic brain atlas of monkeys (*Macaca fuscata*; Kusama and Mabuchi, 1970). The mPFC was divided into three subareas (Dinh et al., 2018); the area anterior to the cingulate sulcus (anterior mPFC), area dorsal to the fundus of the cingulate sulcus (dorsal mPFC), and area ventral to the fundus of the cingulate sulcus (ventral mPFC; **Figure 5**). Consistent with the previous study in the mPFC (Dinh et al., 2018), the snake-responsive neurons were located mainly in the ventral mPFC (**Figure 5**). Especially, the striking-selective neurons were located in the ventral mPFC corresponding to the pregenual part of the ACC (i.e., rACC).

TABLE 1 | Discriminant analyses of the multidimensional scaling (MDS) results in the medial prefrontal cortex (mPFC).

Epochs	Correct ratio (%)	*p*
Epoch 1 (0–50 ms)		
Striking vs. non-striking	56.3	0.127
Epoch 2 (50–100 ms)		
Striking vs. non-striking	100	0.016
Epoch 3 (100–150 ms)		
Striking vs. non-striking	100	0.012
Epoch 4 (150–200 ms)		
Striking vs. non-striking	65.2	0.271

DISCUSSION

In the present study, we showed that the monkey mPFC neurons responded stronger and faster to snakes with striking postures compared with snakes with non-striking postures. Furthermore, population activity patterns of the mPFC neurons discriminated against snakes with striking postures from those with non-striking postures in epochs 2 and 3. Ethological studies reported that the striking speed of snakes is fast enough to preclude motor escape responses from mammal species (Penning et al., 2016). Given that biting strikes are generally preceded by striking postures (Arnold and Bennett, 1984; Greene, 1988), fast discrimination of snake posture may be critical to surviving agonistic encounters with snakes. Consistently, monkeys respond strongly to snake models with striking postures (Etting et al., 2014). We previously reported that mPFC and pulvinar neurons responded stronger and quicker to snakes compared with other animals (Le et al., 2013; Dinh et al., 2018), and that pulvinar neurons also discriminated against snakes with striking postures from those with non-striking postures (Le et al., 2014). Furthermore, MDS locations of snakes with and without striking postures in the mPFC were significantly correlated with those in the pulvinar, suggesting that the mPFC receives inputs directly from the pulvinar or indirectly *via* the amygdala since the pulvinar also sends projections to the amygdala (Rafal et al., 2015; Diano et al., 2017; Elorette et al., 2018), and the pulvinar and amygdala send projections to the mPFC (Porrino et al., 1981; Romanski et al., 1997). These results suggest that the mPFC and subcortical visual pathways are particularly sensitive to evolutionary threats (striking postures of snakes), which is consistent with the Snake Detection Theory, in which predations of primates by snakes are important selective forces to shape the primate brain to be sensitive to snakes (Isbell, 2006, 2009). These findings support the idea of the evolutionary origin of

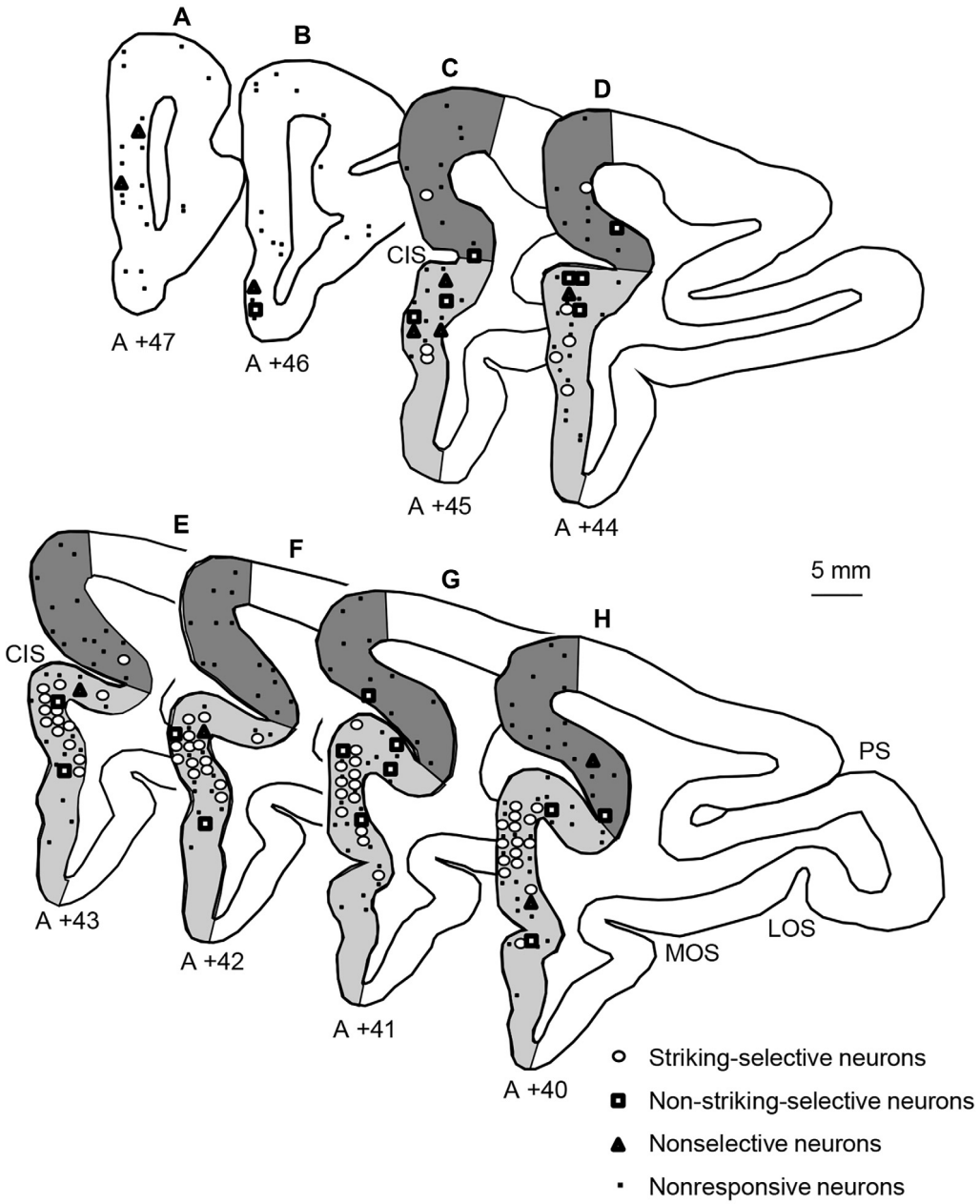

FIGURE 5 | Locations of the three types of snake-responsive neurons in the mPFC. **(A–H)** Locations of mPFC neurons plotted in coronal sections at different A-P levels. The number under each section, distance (mm) anteriorly from the interaural line. Open circles, striking-selective neurons (n = 64); open squares, non-striking-selective neurons (n = 20); open triangles, nonselective neurons (n = 11); dots, nonresponsive neurons (n = 140). The mPFC was divided into three subareas: an anterior part of the mPFC (white areas in **A,B**), a dorsal part of the mPFC (dark gray areas in **C–H**), and a ventral part of the mPFC (light gray areas in **C–H**). PS, principal sulcus; LOS, lateral orbital sulcus; MOS, medial orbital sulcus; CIS, cingulate sulcus.

specific phobia, especially ophidiophobia (see "Introduction" section). The striking-selective neurons were located mostly in the mPFC area roughly corresponding to the pregenual part of the ACC. Human imaging studies reported that phobogenic stimuli elicited activation in the rACC that roughly corresponds to the pregenual part of the ACC (see "Introduction" section), and that functional connectivity between the rACC and amygdala increased (Gold et al., 2015) and activity in the rACC covaried with activity in the amygdala (Carlson et al., 2009) in presence of threat stimuli. Previous electrophysiological studies reported that rACC neurons showed excitatory responses to aversive cues and inhibitory responses to rewarding cues

(Takenouchi et al., 1999; Amemori and Graybiel, 2012) and that microstimulation of the rACC increased bias for avoidance behavior (Amemori and Graybiel, 2012), suggesting that activity in the rACC may be anxiogenic. Consistently, a meta-analysis of imaging studies of patients and at-risk groups suggests that the pregenual ACC is associated with the expression of emotional psychopathology (Marusak et al., 2016). Furthermore, cortical thickness in the pregenual ACC was increased in patients with animal phobia including ophidiophobia compared with healthy controls (Rauch et al., 2004). These findings suggest that the rACC, along with the amygdala, is an important brain region for the development of ophidiophbia.

Attentional bias to specific animals promotes anxiety and phobia (see "Introduction" section). The mPFC is reported to be involved in attentional allocation to various salient visual stimuli (Dalley et al., 2004; Guillem et al., 2011; Kaping et al., 2011; Mao et al., 2017). These findings suggest that neurons in the rACC, which receive inputs from the amygdala and pulvinar, may guide attention to focus on snakes. Consistent with this idea, gray matter volume in the rACC was positively correlated with covert attention to threatening stimuli (Carlson et al., 2012). The increases in attention bias to snakes might be associated with the pathogenesis of ophidiophobia. Human behavioral studies reported that training anxious subjects to guide attention to non-threatening faces from threatening faces reduced anxiety (Heeren et al., 2013). The same method may be applied to patients to treat ophidiophobia. On the other hand, the pregenual ACC (rACC) has bidirectional (reciprocal) connections with the amygdala (Calderazzo et al., 2020), and these bidirectional connections are excitatory (Laviolette et al., 2005; Bissière et al., 2008). It is reported that activity in the rACC is required to develop aversive conditioning in the amygdala (Laviolette et al., 2005; Bissière et al., 2008). These findings suggest that rACC neurons responsive to snakes may guide aversive conditioning in the amygdala. A human behavioral study using aversive conditioning reported that the association between snakes and unconditioned shock was more strongly formed than the association between other control objects and shock (Öhman and Mineka, 2003). These findings suggest that the rACC might initially and preferentially respond to snakes through direct inputs from the pulvinar. The rACC is also likely to facilitate amygdala-dependent conditioning processes, which in turn might further facilitate rACC responses to snakes. The increased activity in the rACC may promote attention to snakes, which may increase the likelihood of developing ophidiophobia. Taken together, these findings highlight the role of the rACC-amygdala circuits in the pathogenesis of specific phobia.

LIMITATION

First, the responses to snakes could depend on low-grade visual features of the snake stimuli, but not on their postures. However, our previous study reported that the responses to snakes in the mPFC were decreased by scrambling of the stimuli using two different methods (quadrate-scrambling, in which snake images were cut into small fragments, and the fragments were randomly reassembled, and Fourier-scrambling, in which shapes were degraded while the global low-level visual features of the original snake images were maintained) (Dinh et al., 2018). These findings suggest that mPFC neurons responded to snake shapes (i.e., snake postures), but not to low-grade visual features of snake images.

Second, the mPFC neurons were recorded from the two monkeys. Since the proportions of snake-responsive and striking-selective neurons among the mPFC neurons were similar between the two monkeys (**Supplementary Table 1**), the data from the two monkeys were combined for further analyses. Although this way of analysis (analyses of combined data of neuronal responses recorded from a few animals) is relatively common in monkey neurophysiological studies (e.g., Tsao et al., 2006; Tsujimoto et al., 2010; Cavanaugh et al., 2020), further studies using more animals are required to test the present hypothesis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethical Committee for Animal Experiments in University of Toyama.

AUTHOR CONTRIBUTIONS

HNishij conceived the study and designed the experiment. HD and QL performed the experiment. QL and HNishij analyzed the data and wrote the article. HNishij, HNishim, JM, TS, RM, CT, and TO revised the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2021.653250/full#supplementary-material.

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REVIEW
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Sleep Disorders in Children With Autism Spectrum Disorder: Insights From Animal Models, Especially Non-human Primate Model

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Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with deficient social skills, communication deficits and repetitive behaviors. The prevalence of ASD has increased among children in recent years. Children with ASD experience more sleep problems, and sleep appears to be essential for the survival and integrity of most living organisms, especially for typical synaptic development and brain plasticity. Many methods have been used to assess sleep problems over past decades such as sleep diaries and parent-reported questionnaires, electroencephalography, actigraphy and videosomnography. A substantial number of rodent and non-human primate models of ASD have been generated. Many of these animal models exhibited sleep disorders at an early age. The aim of this review is to examine and discuss sleep disorders in children with ASD. Toward this aim, we evaluated the prevalence, clinical characteristics, phenotypic analyses, and pathophysiological brain mechanisms of ASD. We highlight the current state of animal models for ASD and explore their implications and prospects for investigating sleep disorders associated with ASD.

Keywords: autism, sleep, non-human primate, brain development, animal model

INTRODUCTION

Sleep appears to be essential for most living organisms' survival and integrity, especially for typical synaptic development and brain plasticity (Fogel et al., 2012; Hartsock and Spencer, 2020). Over the past decades, the role of sleep in learning and memory has been probed by many studies at behavioral, systemic, cellular, and molecular levels (Walker and Stickgold, 2006; Rawashdeh et al., 2007; Sawangjit et al., 2018; Kim et al., 2019). The American Academy of Sleep Medicine (AASM) has recently released the third edition of the International Classification of Sleep Disorders (ICSD-3) in 2014. This guideline grouped sleep disorders into seven basic types: insomnia disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-disordered breathing, movement disorders, parasomnias, and other sleep disorders (Sateia, 2014; Ito and Inoue, 2015). There are universal physiologic changes during sleep, and some

biologic, environmental, psychological, social as well as genetic factors can affect change in the sleep pattern (Jenni and O'Connor, 2005; Bathory and Tomopoulos, 2017). The sleep-wake circadian rhythm is regulated through both circadian and homeostatic processes. Arousal and sleep are active and involved in neurophysiologic processes, including both activation and suppression of neural pathways. Sleep disorders can be an early symptom of the disease, and the presence of rapid eye movement (REM) sleep behavior disorder (RBD) can be used as an early diagnostic indicator for neurodegenerative diseases (Galland et al., 2012; Kotterba, 2015). Besides, sleep disorders have observable effects on physical and mental health of children with autism spectrum disorder (ASD) and their parents (Zhang et al., 2021).

Autism spectrum disorder is a neurodevelopmental disorder and the prevalence of ASD is increasing, with 1 in 59 children in the United States. diagnosed with ASD (Orefice, 2019). ASD is approximately four times more prevalent among males than females (Christensen et al., 2016; Satterstrom et al., 2020). According to the DSM-V, the previous categories of pervasive developmental disorders, pervasive developmental disorder-not otherwise specified (PDD-NOS) and Asperger disorder were combined into ASD. The new diagnostic criteria from DSM-V defined ASD as a heterogeneous spectrum disorder with deficits in social interaction and communication, restricted and repetitive interests, and stereotyped behaviors (American Psychiatric Association, 2013; Devnani and Hegde, 2015).

It is not surprising when considering the numerous health and behavioral issues that sleep disturbance are commonly observed in the clinical progression of ASD. Children with ASD experience more sleep problems compared with the general population, particularly insomnia. Sleep-wake cycle abnormalities are associated with communication deficits, stereotyped behaviors, and autism severity (Tudor et al., 2012). Disrupted sleep may exacerbate the daily dysfunction of ASD children, such as social and communication skills, behavioral performance, stereotypical behaviors, and motor output on non-verbal performance tasks (Schreck et al., 2004; Limoges et al., 2013).

As we gain deeper knowledge of the neural mechanisms of ASD and sleep, more contributions from sleep-related biomarkers to the study of neurophysiology in ASD. Prospectively, the emergence of digital technologies and devices is making studies of sleep physiology more flexible and convenient. The sleep study provided new insights for research on the children with ASD when compared with the other behavioral tests currently used in human subjects and animal experimental models. This review's main objective is to explore animal models' role, especially non-human primate (NHP) models, as a useful tool to investigate sleep disorders in ASD children. Firstly, we present data on sleep disorders in autistic children, emphasizing their prevalence, clinical characteristics, phenotypic analyses, and pathophysiological mechanisms. Next, we highlight the current state of animal models for ASD and explore their implications and future prospects in translational research. We suggest that using NHP animal models may provide insights into sleep disorders in ASD.

THE ROLE OF SLEEP

In most mammalian species, sleep amounts are highest during the neonatal period (Weber and Dan, 2016). Sleep loss can significantly affect a child's health-related quality and activities of daily living (Maski and Kothare, 2013). The brain is one of the organs most impacted by sleep or the lack thereof while adolescence is a critical period for brain reorganization. It is beyond doubt that sleep disorders during this period exert irreversible effects on children's brain development (Roffwarg et al., 1966; Klein et al., 2000; Weber et al., 2018). REM sleep can prune newly formed postsynaptic dendritic spines during motor learning (Li et al., 2017), and the balance of newly formed and original dendritic spines is crucial for neuronal circuit development and behavioral improvement in children. Two studies found that sleep enhance cortical plasticity in the visual cortex during the developmental critical period (Frank et al., 2001; Aton et al., 2009). In conclusion, sleep seems to be important for brain development, learning, and memory consolidation by selectively eliminating and maintaining newly formed synapses (Li et al., 2017).

Sleep deprivation may cause physical diseases and developmental problems. During early life, sleep deprivation has been shown to have long-term implications for social behaviors in adulthood (Hudson et al., 2020). Neural substrates can be affected by sleep deprivation, including the prefrontal cortex, basal ganglia, and amygdala. Furthermore, sleep deprivation may cause difficulties in executive functioning, reward learning as well as emotional reactivity. Such issues may contribute to difficulties in judgment, resolution of problems, challenging behaviors, emotional control, and public health concerns, such as depression, suicide, and risk-taking behavior (Maski and Kothare, 2013). These findings indicate that insufficient sleep during early life has persistent effects on brain development and later behavioral performance.

It has been assumed that sleep can clear out brain's toxins, such as beta-amyloid which was associated with Alzheimer's disease (Xie et al., 2013). Sleep is essential for maintaining the body's physical health and is associated with neurodegeneration, metabolic diseases, cancer, and aging. The processes of growth and development are related to sleep quality. The abnormal sleep and circadian also affect hormones and metabolism (Li et al., 2018a). Getting adequate sleep can help the immune system to better react against infection (Grigg-Damberger, 2009; Herculano-Houzel, 2013; Welberg, 2013).

CLINICAL CHARACTERISTICS OF SLEEP DISORDERS IN ASD CHILDREN

Many neurodevelopmental processes have been reported in the children with ASD, such as synaptic plasticity, neurogenesis and migration of neuron (Gilbert and Man, 2017). About 40–80% of children with ASD exhibit at least one sleep-related problems (Verhoeff et al., 2018), including irregular sleeping and waking patterns, decreased sleep efficiency, reductions in total sleep time and REM sleep time, sleep onset delays, decreased

sleep efficiency, increased wakening after sleep onset, bedtime resistance, and daytime sleepiness (Humphreys et al., 2014). Studies utilizing Actigraphy (ACT) and Polysomnogram (PSG) have found that increased sleep latency, and decreased sleep duration and sleep efficiency in ASD children (Elrod and Hood, 2015). A comprehensive review in children with ASD reported that insomnia is one of the most common sleep problems (Souders et al., 2009). Another study also documented that the predominant sleep disorder included insomnia, difficulty falling, and staying asleep (Malow et al., 2006). Mutluer et al. (2016) found that the most common symptoms reported were troubles falling asleep, sleep after waking up and tired after sleeping.

PREVALENCE OF SLEEP PROBLEMS IN CHILDREN WITH ASD

Childhood sleep disorders which are mostly reported by parents are associated with emotional, cognitive, and behavioral disturbances. Sleep disturbances occur in approximately 20–30% of preschool children, including bedtime resistance, sleep onset delays, night terrors or nightmares, and repetitive rhythmic behaviors (Lozoff et al., 1985; Krakowiak et al., 2008; Knappe et al., 2020). The abnormalities of ASD may predispose children to various threaten of sleep and make them especially susceptible to sleep problems (Morgenthaler et al., 2007; Maxwell-Horn and Malow, 2017). Sleep problems have become one of the most common symptoms among ASD children (Richdale, 1999; Wiggs, 2001; Liu et al., 2006; Uren et al., 2019). Two studies compared sleep behaviors of ASD with typically developing (TD) children, they found that 66% of ASD children exhibited moderate sleep disturbances (Souders et al., 2009) and 71% in another study (Malow et al., 2016). A parent-reported study found that 35% of ASD children had at least one sleep dysfunction (Krakowiak et al., 2008). The risk of sleep disturbance is 2.8-fold higher in children with ASD (Köse et al., 2017). A recent study repeated sleep measures at different age in 5,151 children, and found that ASD children have an increase in sleep problems with age, whereas TD children decrease (Verhoeff et al., 2018).

PHENOTYPE ANALYSES FOR SLEEP DISORDERS

Over the past years, many different sleep analysis methods have been reported (Lomeli et al., 2008; Kelly et al., 2012; Ibáñez et al., 2018; de Zambotti et al., 2019). Infection, pain as well as trauma can disrupt sleep and activity (Vandekerckhove and Cluydts, 2010; Doufas et al., 2012), even some issues that might seem minor to us are often very significant to a child. As children progress from infancy to adolescence, sleep structure, sleep behavior and sleep duration will also change (Williams et al., 2013), it is crucial to take into account the specificity of different ages of children when investigating sleep states. Some sleep studies require an intimate contact of the electrode with the skin and even require surgical implantation of electrodes, which are difficult to apply in freely moving animals and humans, particularly in children related to the lively side of their nature. Even though several new technological developments have been brought to reduce inconvenience, pain, and further damage of these methods, expensive and burdensome must also be considered, especially for long-term studies that include large samples. Some assessments were developed to monitor sleep through observation of body motion and posture. These methods could obviate the need for direct contact and even avoid surgery or electrode implantation. It is non-invasive and low cost. Nevertheless, the behavioral observation does not provide sufficient information compared to those provided by electroencephalogram (EEG) and electromyogram (EMG). In general, both humans' and animals' sleep analyses include sleep patterns, locomotor activity, temperature, and food intake. The current study summarized phenotype analyses for sleep disorders obtained from sleep diaries, parent-reported questionnaires, electroencephalography, actigraphy, and videosomnography. We summarized the main types of experimental approaches applicable to assessment methods of sleep studies and all of these methods have advantages and disadvantages (**Figure 1**). The selection of clinical sleep assessment should be tailored to children's unique characteristics, and safety and feasibility must also be taken into consideration.

Sleep Diaries and Parent-Reported Questionnaires

Subjective measures including sleep diaries and parent-reported questionnaires are the most common analyses in human studies. They have several advantages such as the non-invasive ease of acquisition and low cost. Parents are usually quick to recognize any changes in their child's behavior and mood, and these observations should be recorded (Bhargava, 2011). One of the most common parent-reported questionnaires is Children's Sleep Habits Questionnaire (CSHQ), a parent-report sleep screening instrument designed for school-aged children. The CSHQ score has eight measures, evaluating the behavioral and psychological symptoms of sleep disorders in children (Owens et al., 2000). Many factors affect the reliability of sleep analysis. These factors include the aspect of sleep assessed, the period of sleep aggregated, and the sample population and so forth (Acebo et al., 1999; Camerota et al., 2018). Most retrospective studies on sleep are reported by proxies, such data are limited by problems related to recall bias and subject selection bias (Short et al., 2017). When parents have to estimate the sleep habits of their children, it has been shown that parents tended to estimate with more accurate sleep schedule variables than time awake in bed (Werner et al., 2008). Moreover, the consistency of their reports decreased when the monitoring lasted a long time (Short et al., 2013). Although biases are introduced when utilizing these methods, sleep diaries, and parent reports are commonly used in monitoring children with sleep problems because of their low cost and ease of administration (Honomichl et al., 2002).

Electroencephalography

The influence of technology advances becomes increasingly evident in the study of neuroscience. EEG can provide the

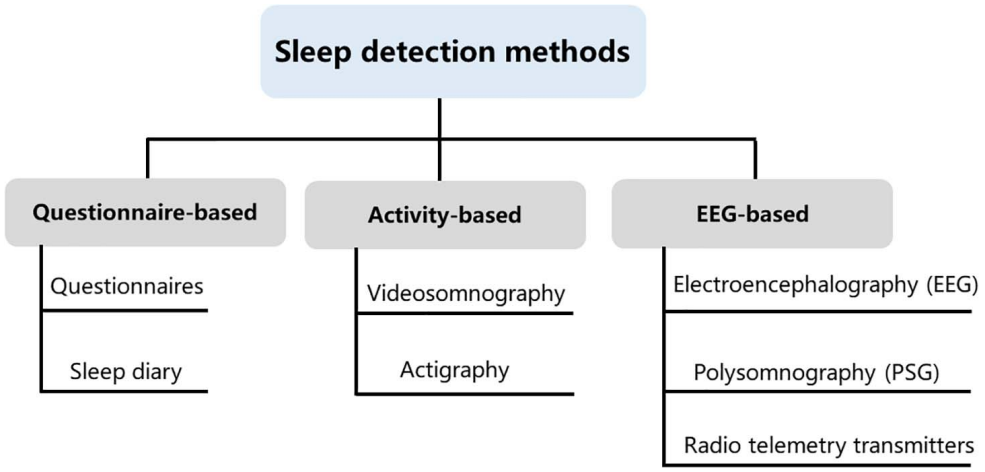

FIGURE 1 | Sleep detection methods. Current methods available for measuring sleep in young children include questionnaire-based, activity-based, and electroencephalogram (EEG)-based methods. These three types of assessments are not interchangeable, as each method contains its own idiosyncrasies that can influence the quality and meaning of the data that are collected.

temporal and spatial characteristics of subject. A sleep EEG is a recording of the electrical activity of the brain while you are awake and then asleep. It involves having small disks (electrodes) which record the activity attached to the subject's scalp (Feinberg et al., 1967; Weber and Dan, 2016). Compared with a single-channel EEG, one technique named polysomnogram (PSG) is considered as the gold standard to objectively assess sleep (O'Donnell et al., 2018). PSG can be used in a diverse range of monitoring, such as brain electrical activity, muscle activity, eye movements, respiratory rate and other channels relying on experimental design (Boulos et al., 2019). It integrates both normal and abnormal physiological indicators of brain electrical activity, sleeps architecture, sleep stages, quality of sleep, eye movements, and physical activities during the sleep period. Wakefulness, NREM sleep, and REM sleep can be clearly distinguished making sleep a directly quantifiable behavior, which could be introduced more easily into clinical routine and less stressful for patients (Blume et al., 2015). The main drawback of PSG is the need of electrodes attached to the skin surface, and not convenient to use in clinical sleep monitoring for children (Lucey et al., 2016). The children cannot be sedated by given medicine such as tranquilizers or sleep aids during the PSG sleep study, and thus doctors may use a blanket or papoose board to keep the child from rolling around on the bed or pulling on the wires. However, this issue may restrict children's normal sleep as we don't expect. And also, PSG instruments are bulky and expensive and may be difficult to monitor changes in patients for long-term studies (Stepnowsky et al., 2013; Qin et al., 2020). Recently, telemetry transmitters have been used for long-term measuring of EEG and electromyography signals in rodent and NHP animals, it could collect data from conscious, freely moving laboratory animals without skin-electrode contact impedance and reduce animals' stress (Ishikawa et al., 2017; Qiu et al., 2019). This strategy can be potentially applied for future clinical applications.

Actigraphy

Actigraphy is a non-invasive method that measures limb movement by a watch-size accelerometer to determine sleep and wake episodes. It allows for multiple-day data collection in natural environments. One study compared the validity of actigraphy and PSG, found that intraclass correlations between PSG and actigraphy variables were strong (>0.80) for sleep latency, sleep duration, and sleep efficiency (Bélanger et al., 2013). Nevertheless, lack of correspondence of circadian sleep-wake cycles between actigraphy and PSG was confirmed in school-age children (Meltzer et al., 2016). Actigraphy assessments may severely underestimate the true sleep statements in children with significantly elevated sleep disorders (Sadeh, 2011).

The next generation multisensory consumer sleep trackers are different from the first motion-based generation of consumer wearables (actigraphy). New generation sleep trackers apply algorithms to achieve functions approximately similar to PSG. Fitbit (Montgomery-Downs et al., 2012; Meltzer et al., 2015; de Zambotti et al., 2016; Mantua et al., 2016; Cook et al., 2017; de Zambotti et al., 2018) and Jawbone (de Zambotti et al., 2015; Toon et al., 2016; Cook et al., 2018) sleep trackers are most frequently tested wearables and their performance has always been compared with PSG. Boe et al. (2019) recently presented a wireless, wearable sensor measuring hand acceleration, electrocardiography (ECG), and skin temperature that outperforms the ActiWatch (one common equipment of actigraphy), detecting wake and sleep with a recall of 74.4 and 90.0%, respectively.

Videosomnography

For centuries, many videosomnography monitoring systems have been used to measure predefined daily activities continuously (von Ziegler et al., 2021). Like actigraphy, the advantages of

videosomnography lie in its objective documentation for long-term interval (Goodlin-Jones et al., 2001; Burnham et al., 2002). It can also be used for capturing abnormal events such as parasomnias during night. However, there are several challenges using videosomnography in sleep research for children. First of all, the portable systems that capture time-lapse video recording are expensive and often need laborious and subjective human labeling. Additionally, camera is placed in fixed positions, the angle of review and the motion of children may affect the quality of video recording. Finally, ethical concerns and privacy issues of videosomnography surveillance system must be considered (Sadeh, 2015; Schwichtenberg et al., 2018). Videosomnography is now widely used in animal sleep research. Most non-invasive rodent sleep assessments depend on gross body movement (Pack et al., 2007; Fisher et al., 2012). Three-state sleep staging can be recorded by using electric field sensors to capture both gross body movement and respiration-related measures (McShane et al., 2012; Mingrone et al., 2020). For NHP study, sleep states are judged by focusing on two major behavioral features: whether the eyes were open or closed, and whether gross movements were present or absent (Prechtl, 1974; Mizuno et al., 2006; Chen et al., 2017). Over the past years, software packages based on deep learning/neural networks allow marker less tracking of multiple, hand-picked body points with astonishing performance.

ANIMAL MODELS USED IN THE STUDY OF ASD

Numerous animal models of ASD have been generated in the last decade (Peñagarikano et al., 2011; Li et al., 2015; Kazdoba et al., 2016; Sacai et al., 2020). Many ASD-associated genes such as Neuroligins play a crucial role in regulation of synaptic adhesion and keeping imbalance between excitatory and inhibitory control in brain circuits (Wintler et al., 2020). The gene editing tools have rapidly been adopted by scientists to parse the role of genetic abnormalities in the etiology and symptomology of ASD. Because the more established gene editing technologies were used in the mice, mice have become the primary animal model of genetic diseases (Crawley, 2012). Growing studies of NHP models have been generated because their close phylogenetic relatedness to humans (Gadad et al., 2013). Moreover, mounting evidence suggests that environmental factors during early development is important. Animal models of maternal exposure to valproic acid and maternal immune activation appear to be the most commonly used. Frequent blood draws and PSG recordings, which are difficult procedures for children with ASD, also make the ASD models becoming ideal candidates. Here, we summarize some rodent (**Table 1**) and NHP (**Table 2**) models of ASD, which may have potential value to investigate the causes and effects of ASD, as well as their effects on brain development and sleep disorders.

Rodent Models for ASD

The CNTNAP2 gene encodes cortactin-associated protein-like 2 (CASPR2), which is a cell adhesion molecule and receptor (Jackman et al., 2009). Research of CNTNAP2 demonstrated a connection between genetic risk for autism and specific brain structures (Alarcón et al., 2008). A linkage study reported an increased familial risk for autism with mutations of the CNTNAP2 gene (Arking et al., 2008). Cntnap2 knockout (KO) mice have very similar presentations as with ASD including hyperactivity and epileptic seizures. Analyses of these mice indicated abnormal neuronal migration and synchrony (Peñagarikano et al., 2011).

Neuroligins (NLs) are a diverse class of proteins distributed molecules with functions of excitatory or inhibitory synapse specification (Ichtchenko et al., 1995; Ichtchenko et al., 1996; Graf et al., 2004; Blundell et al., 2010). Neuroligin-1 (NLG-1) is enriched preferentially at excitatory synapses (Song et al., 1999), neuroligin-2 (NLG-2) is enriched at inhibitory synapses (Varoqueaux et al., 2004; Levinson and El-Husseini, 2005), and neuroligin-3 (NLG-3) appears to be present at both (Fekete et al., 2015). The activity of NLG1 is impaired by prolonged wakefulness. Neuroligin-1 is related to neuronal activity and associated with regulation of sleep and wake (El Helou et al., 2013). Janine et al. found that NLG-1 knockout mice can hardly sustain wakefulness and spend more time in NREM sleep. Neuroligin-2 knock-out mice have less total sleep time and exhibit abnormal spike and wave discharges and behavioral arrests characteristic of absence seizures (Cao et al., 2020). Neuroligin-3 knock-out mice exhibit reduced fear conditioning, olfactory impairments and hyperactivity, as well as reduced ultrasound vocalization and social novelty preference (Radyushkin et al., 2009; Liu et al., 2017).

It has been proven that SHANK3 may induce sleep difficulties in patients with ASD. SHANK proteins are important organizers for signaling proteins in the post-synapse of excitatory neurons. In neurons, SHANK2 and SHANK3 have a positive effect on the induction and maturation of dendritic spines, whereas SHANK1 induces the enlargement of spine heads. Patients with an ASD-associated condition called Phelan-McDermid syndrome (PMS) are often missing the SHANK3 gene and they also often have sleep problems (Phelan and McDermid, 2012; Bro et al., 2017; De Rubeis et al., 2018). A recent meta-analysis of SHANK mutations suggested that SHANK3 mutations have a higher frequency and penetrance in individuals with ASD, compared to SHANK1 and SHANK2 (Leblond et al., 2014). Shank3 mutant mice show a variety of features of both ASD and PMS (Jaramillo et al., 2017; Ingiosi et al., 2019). In Shank3 heterozygous mice, there was a reduction in basal neurotransmission (Bozdagi et al., 2010). Shank3 knockout mice exhibit many autistic-like behaviors such as repetitive grooming, social deficits, reduced activity, anxiety-related behavior, as well as learning and memory impairments (Jaramillo et al., 2016; Dhamne et al., 2017). Shank3 KO mice have reduced sleep intensity and delayed sleep onset.

Overall, there were many other rodent models of ASD displaying reduced sleep time: 16p11.2, Fmr1, Mecp2, Ube3a, Rims1, Scn1a, Scn8a, Disc1, Gabrb3,Camk2a, Cacna1g, and Npas2 (Dudley et al., 2003; Lee et al., 2004; Anderson et al., 2005; Lonart et al., 2008; Kimura et al., 2010; Papale et al., 2010; Johnston et al., 2014; Zhou et al., 2014; Ehlen et al., 2015; Kalume et al., 2015; Kumar et al., 2015; Jaramillo et al., 2016;

TABLE 1 | Autism-relevant phenotypes in selected rodent models.

Models	Methods	Ages	Phenotypes	Sleep disorders	Brain development	References
Genetic rodent models	Cntnap2 knockout	7 days to 6 months	Abnormal social contact, hyperactivity and epileptic seizures. Increased repetitive behaviors and reduced juvenile ultrasonic vocalizations	Wake fragmentation and reduced spectral power in the alpha (9–12 Hz) range during wake	Impaired neuron migration and abnormal neural network connectivity	Peñagarikano et al., 2011; Thomas et al., 2017
Genetic rodent models	Neuroligin-1 (NLG1) knockout	2–8 months	Impaired social approach, repetitive behavior and deficits in spatial learning	NLG1 knockout mice do not sustain wakefulness and spend more NREM sleep. Low theta/alpha activity during wakefulness and altered delta synchrony during sleep	Abnormal long-term potentiation in hippocamp and decreased ratio of NMDA/AMPA glutamate receptor at cortico-striatal synapses	Blundell et al., 2010; El Helou et al., 2013
Genetic rodent models	Neuroligin-2 (NLG2) knockout	5–8 weeks	Increased anxiety-like behavior, decreased pain sensitivity, motor coordination, exploratory activity and ultrasonic pup vocalizations. Developmental milestone delays	More wakefulness and less NREM and REM sleep. Abnormal "hyper synchronized" EEG events during wakefulness and REM sleep	Reduced inhibitory synaptic puncta and impaired synaptic neurotransmission	Blundell et al., 2009; Wöhr et al., 2013; Seok et al., 2018
Genetic rodent models	Neuroligin-3 (NLG3) knockout	50–70 days	Reduced fear conditioning. Olfactory impairments and hyperactivity. Reduced ultrasound vocalization and social novelty preference	Significantly impaired EEG power spectral profiles during wake and sleep	Increased inhibitory neuro-transmission in the barrel cortex, enhanced long-term potentiation in the hippocampus. Decrease of total brain volume	Radyushkin et al., 2009; Liu et al., 2017
Genetic rodent models	Shank3 knockout	4–88 days	Repetitive grooming, Abnormal social interactions and vocalizations, and reduced open field activity	Reduced sleep intensity and delayed sleep onset	Impaired long-term potentiation. Impaired transmission and plasticity in hippocampus. Deficits in baseline NMDA receptor-mediated synaptic responses	Jaramillo et al., 2016; Dhamne et al., 2017
Environmentally-induced models	exposure to valproic acid (VPA) during pregnancy	7–40 days	Social behavioral deficits, increased repetitive behavior, and impaired communication	More wake and NREM sleep, disrupt sleep architecture. Decreased theta and increased gamma power during REM sleep	Decreased cortical levels of GAD65 and GAD67—markers of GABAergic synapses. Increased basal levels of serotonin	Tsujino et al., 2007; Nicolini and Fahnestock, 2018
Environmentally-induced models	Pregnant mice infected with virus or synthetic dsRNA, poly(I:C)	7–12 weeks	Reduced social behavior and increased anxiety-like behavior	Abnormal EEG power and spontaneous epileptiform activity	Deficits in synaptic strength of prefrontal to amygdala neural circuits. Increases in microglia and neuro-inflammatory markers	Li et al., 2018b; Missig et al., 2018

TABLE 2 | Autism-relevant phenotypes in selected primate models.

Models	Methods	Ages	Phenotypes	Sleep disorder	Brain development	References
Rett Syndrome	MECP2 mutations mediated by TALENs	7–8 months	Increased sensory threshold and stereotypical behaviors, social communication deficits and abnormal eye-tracking	Sleep in mutants was more fragmented. Significantly longer awake durations and shorter total sleep durations	Significantly reduced cortical gray matter and white matter. Reduced total cortical volumes and thicknesses	Chen et al., 2017
MECP2 duplication syndrome	MECP2 overexpression by lentivirus-based transgenic	12–18 months and then to 55 months	Increased repetitive behavior and stress responses. Reduced social contact	N/A	Reduced β-synchronization within frontal-parieto-occipital networks. Hypoconnectivity in prefrontal and cingulate networks	Liu et al., 2016; Cai et al., 2020
Maternal immune activation	Poly IC injection	6–24 months	Increased repetitive behaviors, communication deficits, abnormal social interactions and affiliative calls	N/A	Altered dendritic morphology. Reduces in both gray matter and white matter. Alterations of dendritic morphology	Bauman et al., 2014; Machado et al., 2015
Maternal immune activation	Valproic acid (VPA) explored	17–21 months	Abnormal social interaction, increased stereotypies, and abnormal eye-tracking	N/A	Severe neurogenesis defects and abnormal neurogenesis	Zhao H. et al., 2019
SHANK3 mutation	CRISPR/Cas9	1–12 months	Motor deficits and increased repetitive behaviors. Social and learning impairments	Increased sleep latency and nocturnal waking. Reduced sleep efficiency	Decreased gray matter. Dysregulated resting-state brain connectivity	Zhou et al., 2019

Tatsuki et al., 2016; Dittrich et al., 2017; Lu et al., 2019). Although the majority of these mutant rodent models exhibit reduced activity, which could be indicative of decrease sleep duration, the prevalence of serious sleep problems such as sleep fragmentation is far less than what has been observed in the clinical population.

While there is strong genetic effect, the etiology of ASD seems to be multifactorial. Environmental factors including toxins, pesticides, infection, and drugs also have a strong correlation. Environmental exposure during preconception, prenatal, and postnatal pregnancy can impact the immune system and the developing nervous system, and may cause neurodevelopmental disorders including ASD.

Valproic acid (VPA) is a drug used in humans primarily for epilepsy and seizure control. VPA is currently considered to be a risk factor for ASD and is also known teratogenicity (Balfour and Bryson, 1994). It has been demonstrated that exposure to VPA during pregnancy would increase the risk of autism in children based on several studies in humans (Laegreid et al., 1993; Christianson et al., 1994) and experimental evidence in animals (Lin et al., 2013). Furthermore, rodents prenatally exposed to this drug exhibit autism-like behavior including social behavioral deficits, repetitive and stereotypic behaviors, and impaired communication (Mychasiuk et al., 2012; Nicolini and Fahnestock, 2018). Intraperitoneal injection of VPA to rats with pregnancy would make their offspring exhibiting autism relevant behavioral and physiological indicators (Schneider et al., 2008).

Several studies have reported correlation between maternal antibody reactivity toward fetal brain proteins and ASD in the children (Braunschweig et al., 2008; Croen et al., 2008; Brimberg et al., 2013). In the rodent maternal immune activation model of ASD (Smith et al., 2007; Malkova et al., 2012; Choi et al., 2016; Kim et al., 2017), offspring from pregnant mice which were infected with virus or injected intra-peritoneally with synthetic dsRNA [poly(I: C)], exhibited behavioral symptoms such as social deficits, communication deficits, and repetitive behaviors. For brain neuropathology, the offspring of maternally infected mice displayed significantly fewer Purkinje cells. These data are quite similar to both ASD behavioral and neuropathological phenotypes.

Non-human Primate Models

Non-human primates are among the optimal animal models, in large part because of their close phylogenetic relatedness with humans (Zhang et al., 2014; Nunn and Samson, 2018). With the rapid advances in gene-editing technologies, researchers have established several NHP models for ASD (Liu et al., 2016; Sato et al., 2016; Chen et al., 2017; Tu et al., 2019). It would be valuable

for researchers to be attentive to study of many kinds of disease by using NHP animal models (Anderson, 2000).

MECP2 duplication syndrome is an X-linked recessive syndrome resulting from abnormal genomic rearrangement. The two major clinical symptoms are intellectual disability and anxiety. MECP2 overexpressed monkey models exhibited characteristic features of ASD such as social deficits, repetitive behaviors, and increased anxiety (Liu et al., 2016). Cai et al. reported a combination of gene-circuit-behavior analyses, including MECP2 co-expression network, locomotive and cognitive behaviors, and EEG and fMRI findings in MECP2 overexpressed monkeys. Whole-genome expression analysis revealed MECP2 co-expressed genes were significantly enriched in GABA-related signaling pathways, whereby reduced β-synchronization within frontal-parietal-occipital networks was associated with abnormal locomotive behaviors (Cai et al., 2020).

Rett syndrome caused by mutations in MECP2 is a prototypical neurodevelopmental disorder. Researchers demonstrated that MECP2 mutant monkeys could well mimic autism-associated abnormalities in physiology and social behavior (Chen et al., 2017). The mutant monkeys exhibited significantly increased total awake time and more fragmental sleep during night, which have also been found in Mecp2 mutant mice (Li et al., 2015).

Feng et al. used CRISPR/Cas9 to generate SHANK3, a top autism gene mutant monkey. SHANK3 mutant monkeys tend to be less active and have troubles sleeping that they take longer time to fall asleep and wake up more often. Monkeys in this study have severe repetitive movement, deficient social skills, and show brain-activity patterns similar to those seen in autistic people (Le Bras, 2019; Tu et al., 2019). SHANK3-deficient monkeys showed reduced spine density and impaired development of mature neurons in the prefrontal cortex (Zhao et al., 2017). It has also been found that some rhesus macaques carried spontaneous mutation of SHANK3 (Vegué and Roxin, 2015). Spontaneous mutations in NHPs may have the potential to be used as a suitable animal model to figure out the relationships between genetic variants and behaviors (Haus et al., 2014; Zhao et al., 2018).

Rodent animal models of maternal exposure to VPA provided evidence that environmental risk factors in ASD. Recently, Zhao H. et al., 2019 reported the neurodevelopmental and behavioral outcomes of maternal VPA exposure in NHP for the first time. Offspring from maternal exposure to VPA has significantly impaired neuronal development. VPA-exposed monkey offspring showed impaired social interaction, communication disabilities, and abnormal eye-tracking (Zhao H. et al., 2019).

When rhesus monkeys were given the viral mimicking synthetic double-stranded RNA (polyinosinic:polycytidylic acid stabilized with poly-L-lysine) during pregnancy, and their offspring could exhibit abnormal repetitive behaviors, altered communication, impaired social interactions and abnormal gaze patterns to salient social information (Bauman et al., 2014; Machado et al., 2015). These offspring with autism-like behaviors also have reduced gray matter in most of the cortex and decreased white matter in the parietal cortex (Short et al., 2010). Novel evidence implicating MIA exposure with alterations of NHP dendritic morphology have been found (Weir et al., 2015). The mother and the fetus exploit several mechanisms in order to avoid fetal rejection and to maintain an immunotolerant environment during pregnancy. The placenta is an important organ that facilitates nutrient exchange. It has been reported that the anatomy of the placenta is varied across species, and it is highest in humans, intermediate in rhesus macaques, and minimal in rodents (Carter, 2007). Thus, the role of the NHP animal model in this field of research is important.

MONKEYS AS AN IDEAL ANIMAL MODEL FOR STUDYING SLEEP IN ASD

An ideal animal model of human disease should show tight junctions with clinical characteristics of the disease. The statistics from United States government in 2010 indicated that almost 90% of the laboratory animals used in science research are mice, rats, and other rodents. NHP only represents 0.28% among all animals (Phillips et al., 2014). However, rodents diverged from humans by more than 70 million years of evolution. There are significantly evolutionary differences in brain anatomy, cognitive capacity, and social behavior between humans and rodents (Kumar and Hedges, 1998; Gibbs et al., 2004). Compared with rodents, rhesus macaque (Macaca mulatta), most common NHP used in study, are separated from humans approximately 25 million years ago and are more similar to humans in genetics, neurobiology, and behavior. Thus, NHP have reasonable behavioral correlates to the characteristics of patients in ASD, such as repetitive behaviors, communication deficits, and stereotyped behavior (Watson and Platt, 2012; Parker et al., 2018). As mentioned previously, prenatal environment and gestational timing may impact neurodevelopment of offspring. The gestational period of rhesus monkeys (165 days) and humans (280 days) is much longer than mouse (18–23 days) (Clancy et al., 2001). Besides, the prenatal immune challenge and neuron development of primates occur mostly during the third trimester of prenatal and during early postnatal period (Careaga et al., 2017). The mouse is becoming increasingly popular for genetic studies. However, the mouse's brain weighs a few grams, and ours weighs one and a half kilos. Can we use the mouse to learn something about our brain? The region of the neocortex is almost 80% in the human brain, which is just 28% in the rat (Roberts and Clarke, 2019). Human prefrontal cortex includes granular and agranular cortex, while rat prefrontal cortex only contains agranular cortex (Ongür and Price, 2000; Uylings et al., 2003). It has been proposed that the prefrontal cortex has a substantial role in social processing, and its potential dysfunction may cause ASD (Sugranyes et al., 2011). The temporal lobe is a morphological brain region which is unique to primates (Colombo et al., 2000; Bryant and Preuss, 2018). The major areas of the human brain classified by Brodmann have also been identified in NHP. Structure and function of the amygdala are nearly the same in the human and non-human primate (Gowen et al., 2007; Rutishauser et al., 2015; Schumann et al., 2016), but remarkably different from the rodent brain (Chareyron et al., 2011). The close relationship of development and evolution between NHP and

human show that great prospects to mimic clinical realities by designing NHP animal models.

As mentioned previously, sleep problems in children with ASD are caused by multi-factorial risks such as abnormal neurodevelopment and environmental factors (Bourgeron, 2007; Owens and Mindell, 2011). Modeling clinical disorders in animals provide an opportunity to improve translational research although the human disorder's clinical phenotype is complex and heterogeneous and lacks objective homologous endpoints across species (Missig et al., 2020). Many previous studies of ASD animal models exhibited several hallmark features which have been documented in humans.

Sleeping studies in humans must be done in accessible samples, predominantly saliva or blood, and confounded by environmental factors. Species-specific differences including light and biological rhythm, as well as sleep features have been noted in studies of sleep (Campbell and Tobler, 1984). For example, rodents are commonly thought to awake during the dark phase and asleep during the light phase. However, researchers found that mice are not explicitly nocturnal, and they have diurnal feeding activity. Researchers also reported that seasonal influences were demonstrated to be more potent on activity than specific genes which was generally considered to control sleep (Daan et al., 2011). Effective use of animals to study normal sleep and sleep disorders must consider known similarities and differences between human and animals. Likewise, sleep is important to keep health and can significantly influence daily activity schedules in NHP (Fruth et al., 2018; Qiu et al., 2019). Sleep structure and EEG patterns of NHP are closely related to the consolidated and monophasic organization observed in humans (Reite et al., 1965; Hsieh et al., 2008), which contrasts with the more fragmented sleep patterns in rodents (Fifel and Cooper, 2014) (**Table 3**). NHP is also a diurnal animal to better recapitulate clinical conditions with behavioral and metabolic properties closer to humans. Humans pass through 4–6 cycles of NREM and REM within a night's sleep, which are much shorter in rats and mice (Fuller et al., 2006; Toth and Bhargava, 2013). Nunn and Samson compared sleep patterns in 30 different species of primates, including humans. Most species generally sleep between 9 and 15 h, while humans averaged just 7 h (Nunn and Samson, 2018). In summary, measuring behavioral and sleep states in NHP may provide a better understanding of sleep disorders in children with ASD compared with rodents.

CURRENT CHALLENGES

Autism spectrum disorder is a neurodevelopmental disorder and the origins of ASD remain unresolved. The potential estimates including genetic, maternal, and environmental effects (Bai et al., 2019). At first, there are various types of genetic variation, such as single-nucleotide polymorphism (SNP) or rare genetic mutations (Weiner et al., 2017). Animal model studies have shown that the impact of genetic on behavior is complex and not completely correspond to specific behavior. Brain development can be influenced by not only the expression of genes, but also modified by environmental factors during the pregnancy and postnatal period. Therefore, the application of gene editing technology in animal models of disease may not completely mimic clinical phenotypes of humans. Secondly, although the NHP animal model has been used to study the impact of environmental modifications on the brain development. Genetic influences may also affect individual responses to different situations and different types of environmental challenges (Machado and Bachevalier, 2003). In this area, rodent models may be more appropriate which have more identical genetic backgrounds compared with NHPs. Besides, NHP exhibit significant and stable individual differences in social commination (Capitanio, 1999; Capitanio and Widaman, 2005).

Non-human primates have much longer reproductive cycle and lower reproduction efficiency compared with rodents, which may bring the difficulties to prepare an adequate quantity of experimental animals. Furthermore, the giant body size of NHP may cause a significant challenge for experimental design. Limitations associated with the gene-editing technique, including editing efficiency, chimeras, and off-target effects, should also be brought to attention (Niu et al., 2014; Chen et al., 2016). Lastly, NHP, rather than other animals, require more significant ethical consideration because its significant cognitive capacity and complex social behavior. Researchers have moral responsibility to ensure that experimental animals receive reduced negative effects and suffering (Bentham, 1996;

TABLE 3 | Different sleep pattern between human and animals.

	Human	Monkey	Rat	Mice
Primary circadian sleep phase	Dark	Dark	Light	Light
Sleep pattern	Monophasic or diphasic	Monophasic or diphasic	Polyphasic	Polyphasic
Total sleep duration (24 h)	6–8 h	9–12 h	12–15 h	12–15 h
Sleep efficiency (%) (12 h dark)	95%	88%	55%	33%
REM sleep (%) (12 h dark)	20–25%	28%	7–9%	3–5%
NREM sleep (%) (12 h dark)	60–83%	76–80%	26–30%	22–29%
References	Roffwarg et al., 1966; Campbell and Tobler, 1984; Carskadon and Dement, 2005	Hsieh et al., 2008; Authier et al., 2014; Rachalski et al., 2014; Ishikawa et al., 2017; Qin et al., 2020	Zepelin et al., 1972; Seelke and Blumberg, 2008	Vyazovskiy et al., 2006; Hasan et al., 2012

Beauchamp and Frey, 2011). Animal experiments should follow the principles of the 3Rs, including replacement, reduction, and refinement (Russell and Burch, 1959; Jennings et al., 2009).

CONCLUSION AND PERSPECTIVES

Autism spectrum disorder is a neurodevelopmental disorder and with the increasing incidence of ASD, it is essential to understand what has changed in our genes and environments that may contribute to these disorders. It has been showed that ASD is not a single disease, but rather several conditions including genetic, maternal, and environmental effects that ultimately cause similar behavioral impairments. The abnormalities of ASD may predispose children to various threaten of sleep and make them especially susceptible to sleep problems. Sleep disorders have been reported as one of the most common symptoms and in up to 80% of children with ASD have sleep problems which may even contribute to the altered brain structure and activity (Blakemore et al., 2006). Thus, understanding how sleep affected children with ASD by specific mechanisms such as brain development and synaptic plasticity will enable a broader understanding of the disorders' causes and provide insights into specific treatments. Over the years, many different sleep analysis methods have been reported. The selection of sleep assessment method should be tailored to specific subjects and taken into consideration of their unique characteristics.

Animal models hold great potential values to investigate the causes and treatments for sleep problems in children with ASD. Numerous animal models of ASD have been generated in the last decade. An ideal animal model should show tight junctions with clinical characteristics of the disease. Rodents are the most common experimental animals and growing studies of NHP models have been generated because their close phylogenetic relatedness to humans.

Because of the complexity and heterogeneity of the ASD, it is still inadequate to understand how genes control and influence complex behavior. The animal models of ASD are currently oversimplified and have many issues. Recently, Liu et al. (2019) demonstrated that an approach to generate cloned monkeys by somatic cell nuclear transfer (SCNT), which can creatively solve the problem of generating NHP models with uniform genetic backgrounds. This study is profoundly improving the overall reproducibility of the model. Continued research used this technique to generate five BMAL1 knockout monkeys for sleeping study, and these monkeys exhibited more activities and reduced sleep during night (Qiu et al., 2019). The development of genome editing technologies (such as CRISPR/Cas9 and base editing, etc.) has opened up the revolutionary ways to directly target and modify genomic sequences in animals (Kang et al., 2019; Zhao J. et al., 2019; Li et al., 2020). We anticipate greater numbers of applications will materialize shortly, such as genome-edited NHP combined with SCNT. Although some issues still need to be solved, studying the sleep disorder across multiple biological scales can offer the hope in the field of translational medicine for ASD and other human diseases. The role of NHP animal model in this process is irreplaceable and must be recognized.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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The Perception of the Müller-Lyer Visual Illusion in Schizophrenics and Non-human Primates: A Translational Approach

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The Müller-Lyer Illusion (MLI) has been suggested as a potential marker for the perceptual impairments observed in schizophrenia patients. Along with some positive symptoms, these deficits are not easily modeled in rodent experiments, and novel animal models are warranted. Previously, MK-801 was shown to reduce susceptibility to MLI in monkeys, raising the prospects of an effective perception-based model. Here, we evaluate the translational feasibility of the MLI task under NMDA receptor blockage as a primate model for schizophrenia. In Experiment 1, eight capuchin monkeys (*Sapajus* spp.) were trained on a touchscreen MLI task. Upon reaching the learning criteria, the monkeys were given ketamine (0.3 mg/kg; i.m.) or saline on four consecutive days and then retested on the MLI task. In Experiment 2, eight chronic schizophrenia patients (and eight matching controls) were tested on the Brentano version of the MLI. Under saline treatment, monkeys were susceptible to MLI, similarly to healthy human participants. Repeated ketamine administrations, however, failed to improve their performance as previous results with MK-801 had shown. Schizophrenic patients, on the other hand, showed a higher susceptibility to MLI when compared to healthy controls. In light of the present and previous studies, the MLI task shows consistent results across monkeys and humans. In spite of potentially being an interesting translational model of schizophrenia, the MLI task warrants further refinement in non-human primates and a broader sample of schizophrenia subtypes.

Keywords: geometric illusion, glutamate model for schizophrenia, sensory integration, non-human primate, schizophrenia

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INTRODUCTION

In spite of being consistently associated with schizophrenia, sensory and perceptual deficits are not considered as core symptoms nor are they used to classify its subtypes. The heterogeneity of the spectrum is mostly defined by the presence of positive or negative symptoms (Khoury et al., 2017). Animal and clinical research literature, on the other hand, has given considerable attention to these impairments and their potential as biomarkers in patients, particularly in the case of sensory gating (e.g., Swerdlow et al., 2006) and visual illusions (e.g., Pessoa et al., 2008; King et al., 2017). Susceptibility to the Müller-Lyer illusion (MLI) is a promising strategy as it has been reported to vary among schizophrenic patients (Letourneau, 1974; Parnas et al., 2001). There are several indications that the MLI may be influenced by top-down modulation from the anterior cingulate (Qiu et al., 2008) and posterior parietal cortex (Weidner and Fink, 2007; Maddaluno et al., 2019). Indeed, these areas are subject to loss of volume or gray matter or even reduced connectivity in schizophrenia patients (Rimol et al., 2010; Roiser et al., 2013). Since these morphometrical changes may intensify as a function of time, sensitivity to the MLI may vary according to the stage of the illness (Parnas et al., 2001).

As a tool for animal model research, sensitivity to the MLI seems to be remarkably conserved across species. It has been identified in primates (Suganuma et al., 2007; Tudusciuc and Nieder, 2010), birds (Warden and Baar, 1929; Winslow, 1933; Pepperberg et al., 2008), and even fish species (Sovrano et al., 2016). Also, the degree of sensitivity, measured in visual angle, seems to be quite similar between naïve monkeys and healthy humans (Tudusciuc and Nieder, 2010). These reports indicate that the MLI may be an illusory byproduct stemming from fundamental features of visual organization and/or processing. It also translates into a stable parameter for cross-species comparisons using similar behavioral tasks.

Few pharmacological models of schizophrenia have been used so far to investigate sensory impairments in nonhuman primates. NMDA receptor blockade by MK-801 increases prepulse inhibition in monkeys (Saletti et al., 2015, 2017), similar to results found with rodents (Gomes et al., 2014). Although sensory gating protocols are commonplace with rodents, visual impairment experiments are not quite as feasible. A translational model of these alterations would, therefore, be more suitable in nonhuman primates. Previously, we introduced a new research protocol in capuchin monkeys to test the effects of NMDA receptor blockade on their sensitivity to MLI (Jacobsen et al., 2017). At very low doses, MK-801 improved performance on the MLI task, an indication of reduced sensitivity to the illusion effect. Increased doses of MK-801, however, are liable to ataxia which would by itself impair the motor performance required on the task. This would preclude a possible dose-dependent sensitivity curve in the MLI. Here, we attempted an alternative strategy of NMDA receptor blockade by means of ketamine. As a drug with approved clinical use both in veterinary and human medicine, its profile may foster easier comparisons between human and nonhuman results. Furthermore, we tested a novel MLI task on chronic schizophrenic patients. The results are discussed in light of using the MLI task as a translational model of perceptual changes in schizophrenia.

MATERIALS AND METHODS

Experiment 1: Effects of Ketamine on MLI Task (Capuchin monkeys)

Subjects

Five adult (10–20 years old) capuchin monkeys (*Sapajus* spp.) were used in this study, four females and one male, weighing between 2 and 5 kg. They were housed in pairs or triads at the Primate Center of the University of Brasilia, Brazil, with home-cages (4 m long, 2.9 m wide, and 2 m high) being provisioned with natural substrate, rope swings, and nest boxes. The animals were tested in their own home-cages under natural light and temperature conditions. They were separated from the rest of their group only during the training and test sessions (see "Procedure" section below). No head or body restrain was enforced. All subjects had prior experience with touchscreen monitors, yet none had been previously exposed to the drug tested. The capuchins had free access to food and water, except during the experimental sessions. All the procedures in the animal experiments were approved by the Animal Ethics Committee of the University of Brasilia (46077/2014) and complied with the Brazilian regulations for the scientific use of laboratory animals (Lei Arouca 11.794/2008), as well as the CONCEA/Brazil and NIH/USA guidelines for the care and use of laboratory animals.

Apparatus and Computer Program

A laptop (Lenovo®, Intel Core i7®, Brazil) connected to a 15 in. touchscreen monitor (Elo Touchsystems®, Brazil) was used for data collection. The apparatus was set up in front of the cage's wire mesh door, about 20 cm from the animal. The area behind the monitor was covered with a black cloth to reduce visual distractions. All training and test tasks were created and run using the E-Prime 2.0 software (Psychology Software Tools Inc®, USA), with the subject's response accuracy being recorded on each session.

Procedure

The training procedures were held 5 days a week, between 8 and 12 AM, being divided into three stages: an initial training phase; the determination of each subject's Point of Subjective Equality (PSE) with and without arrowheads; and lastly a test stage. On all three stages, 5 mm thick straight horizontal black lines on a white background were used as stimuli. These lines were 20–105 mm in length (4–20 visual degrees), with or without arrowheads at the extremities. When present, the length of the arrowheads was 25% of the length of the respective line, forming a 45° angle for outward-pointing arrowheads and 135° for inward-pointing arrowheads. Correct and incorrect responses from the subjects on each task were immediately followed by distinct (0.5 s) buzzer tones. Each correct response was also rewarded with a raisin provided manually to the subject by one of the experimenters.

Training

Subjects were trained for several weeks to select the shortest line in each stimulus pair (with or without arrowheads) presented on the touchscreen monitor (**Figures 1A,B**). Subsequently, the point of subjective equality (PSE) was determined for each animal. The PSE was operationally defined as the difference whereby the subject's performance reached chance level.

Point of Subjective Equality

PSE without arrowheads: we initially calculated the percentage of correct responses required per session using a binomial test with a 95% confidence interval limit (CI) of a random performance (50%) based on the total number of trials per session. As each session consisted of 60 trials (plus five "warm up" trials not used for any analysis), the upper CI was set at 63.19% per session, i.e., 38 correct responses in 60 trials. On any given session, the length difference between the two lines presented was the same on all trials (**Figure 1A**, top). On the subject's first session, the pair of lines had a 50% length difference (140/70 mm). If the subject attained the calculated percentage of correct responses on this session, the difference in length between the two lines was reduced by 10 percentage points (p.p.) on the next session (e.g., 50–10 = 40%). If the criterion was not reached within the first session, the same length difference was repeated on the subsequent session. If the criterion was still not reached, the subject's third session tested the last length difference it had achieved the upper CI minus 2 p.p. For instance, if the upper CI was attained at 50% but not at 40% difference, the next session would use a 48% (50%–2 p.p.) length difference between lines. The procedure was then repeated with successive 2 p.p. decrements at each session until the subject failed to reach the upper CI on two consecutive sessions. The last length difference in which the calculated percentage of correct responses was reached was set as the subject's PSE without arrowheads (e.g., if it failed at a 44% difference, then "PSE without arrowheads" for this subject would be set at 46% length difference between lines).

PSE with arrowheads: In this phase, each session consisted of 60 trials: 30 with "illusion pairs" and 30 with "neutral pairs". For the latter, the direction of the arrowheads accentuated the length difference between the two lines (**Figure 1A**, bottom left), whereas it decreased that of the "illusion pair" (**Figure 1A**, bottom right). Only "illusion pairs" were used to determine each subject's PSE with arrowheads and as such the upper CI was set at 68.7% per session (21 correct responses in 30 trials). A given length difference was tested on four consecutive sessions, held at 24 h intervals. The first line pair tested was the 140/70 mm, corresponding to a 50% difference in length between the two lines. If the subject attained the upper CI on all four sessions, the subsequent four sessions used a pair of lines with a 10 p.p. decrease in length difference (e.g., 50–10 = 40%). If the criterion was not reached on any of these four sessions, the next four-session sequence tested the last difference the subject had achieved the upper CI minus 2 p.p. [e.g., if upper CI was attained at 50% but not at 40% difference, then the next session used 48% (50%–2 p.p.) length difference between the lines]. This procedure was then repeated on the subsequent four-session sequences, in which successive 2 p.p. decrements in length difference were tested until the subject failed to reach the upper CI on any of the sessions. The last length difference in which the upper CI was reached was set as the subject's individual PSE with arrowheads (e.g., if failed at 44% difference, then "PSE with arrowheads" for this subject would be set at 46% length difference between lines).

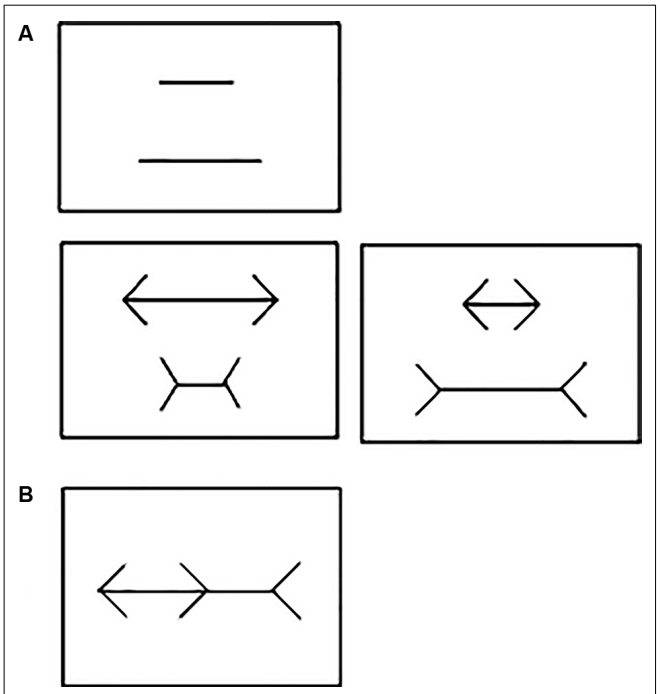

FIGURE 1 | (A) Example of the stimuli used during training, point of subjective equality (PSE) and drug administration sessions in Experiment 1. No arrowheads (Top); neutral pair with arrowheads (Bottom left) and illusion pair with arrowheads (Bottom right). **(B)** Brentano version of the Müller-Lyer Illusion (MLI) used in Experiment 2.

Test

The drug challenge comprised two four-session sequences, held at 24 h intervals. The subjects were captured in their home-cages and received a vehicle or ketamine (0.3 mg/kg, i.m.) injection, respectively, 25 min prior to behavioral testing. Ketamine was dissolved in saline solution and injected in a volume of 0.5 ml/kg (this concentration was determined to reduce muscular pain and to reduce the duration of restraint). Saline was also used as the vehicle control. All sessions at this stage were carried out using the subject's own PSE (with arrowheads) determined in the preceding stage. Each session consisted of 60 trials, 30 with "illusion pairs" and 30 with "neutral pairs". Once again the number of correct answers was determined solely on the responses for the "illusion pairs". "Neutral pair" trials were employed as task controls and therefore a 90% level of correct responses per session in these trials was set as a criterion to ensure that subjects were still following the task rule (i.e., choosing the

shorter line) or that "illusion pairs" were not being chosen randomly.

Experiment 2: Brentano MLI Test in Schizophrenic Patients

Participants

Eight chronic schizophrenia patients (all males) were inpatients recruited at the São Vicente de Paulo Hospital (Federal District, Brazil). The demographic characteristics of each patient are shown in **Table 1**. To be included in the study, patients had to be between 18 and 65 years of age and diagnosed with chronic/residual schizophrenia (ICD: F20.5). Exclusion criteria included: (1) any history of Traumatic Brain Injury; (2) history of a neurological disorder; and (3) current substance abuse or dependence disorder (within the past 6 months). The control group (three females; five males) was recruited to match the patient group demographics. All participants or their legal representatives were required to sign a Free and Informed Consent form prior to the start of procedures. This experiment had been previously approved by the University of Brasília Research Ethics Committee (CAAE 96510318.3.0000.0030).

Apparatus and Procedures

A laptop (Sony® Vaio®, Brazil) connected to a 15" monitor (Bematech®, USA) was used for data collection. All training and test tasks were created and run using the E-Prime 3.0 software (Psychology Software Tools Inc®, USA). The test stimuli consisted of the Brentano version of the MLI (**Figure 1B**). The main axis of the illusion had 20 cm of length (approximately 20 visual degrees) with one fixed arrowhead at each end pointing in the same direction (the direction for each trial was randomly assigned). A third arrowhead pointing in the opposite direction was placed on the main axis between the other two arrowheads. The tip of this central arrowhead was used as the spatial reference to divide the main axis into two segments. All arrowheads subtended a 90° angle formed by two 4-cm long lines. For each experimental session, the participants sat comfortably in front of the screen, kept at a 50-cm distance from their eyes. Each trial started with the presentation of a blank screen for 2 s followed by a tone buzzer. After that, the Brentano MLI stimulus was presented in the center of the screen. On each trial, the central arrowhead would randomly appear at any point on the main axis, between the two other arrowheads. The participants were asked to slide the central arrowhead along the main axis, using the keyboard arrows, so that its tip would divide the main axis into two segments of equal length. The illusory effect of the arrowheads was determined by the distance between the actual center of the axis (i.e., 20 cm) and the participant's estimation. When this had been completed, the participant would press "space bar" and a new trial would start immediately. Each participant was submitted to a total of 20 trials.

At the end of the illusion testing session, the patients (but not control participants) were subjected to a brief version of the PANSS (Positive and Negative Syndrome Scale). The scale was administered by a trained psychiatrist and took approximately 30 min.

Statistical Analysis

For the PSE stage, the difference in length between the pair of lines with and without arrowheads was compared using a paired *t*-test. For the Test stage, the percentage of correct responses on the four-session sequence within each treatment was averaged. Mean values were then analyzed using a two-way analysis of variance (ANOVA) with repeated measures on treatment (vehicle and ketamine) and stimuli pair type ("illusion pair" and "neutral pair"). *Post hoc* comparisons were performed whenever appropriate using Tukey's test. For the human experimental data, schizophrenic and control performance was compared using unpaired *t*-test. Data are presented as the mean PSE percentage or mean percentage of correct answers ± standard error of the mean (SEM). The significance level for all tests was set at $p < 0.05$.

TABLE 1 | Demographics: schizophrenic patients (top) and controls (bottom).

Patient#	Sex	Age	Length of illness (years)	Age of diagnosis	Drugs in use	Highest school level completed	Family mental disease
1	M	59	41	18	Clozapine	Middle school	Yes
2	M	48	31	17	Clozapine + Haloperidol + Lithium	Elementary School	No
3	M	40	13	27	Clozapine + Valproic acid	High school	No
4	M	36	12	24	Clozapine + Olanzapine	Middle school	No
5	M	31	10	21	Haloperidol	Elementary school	No
6	M	37	17	20	Clozapine	High school	No
7	M	23	4	19	Clozapine	Middle school	No
8	M	37	11	26	Clozapine	Elementary school	Yes
Control#	**Sex**	**Age**	**Length of illness (years)**	**Age of diagnosis**	**Drugs in use**	**Highest school level completed**	**Family mental disease**
1	F	23				High school	No
2	M	34				Middle school	No
3	M	28				High school	No
4	M	23				High school	No
5	M	27				High school	No
6	F	40				Elementary school	No
7	F	26				High school	No
8	M	28				High school	No

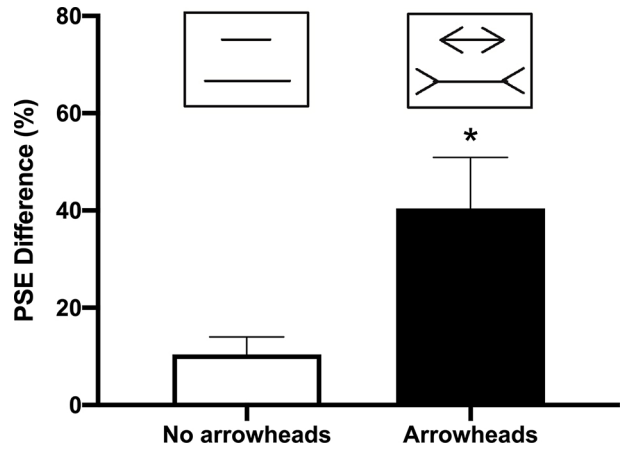

FIGURE 2 | Difference in the point of subjective equality (PSE) with and without arrowheads. Bars indicate the average of the length difference between the two lines obtained when determining the PSE with and without arrowheads (mean + SEM; $n = 5$; $^*p < 0.05$).

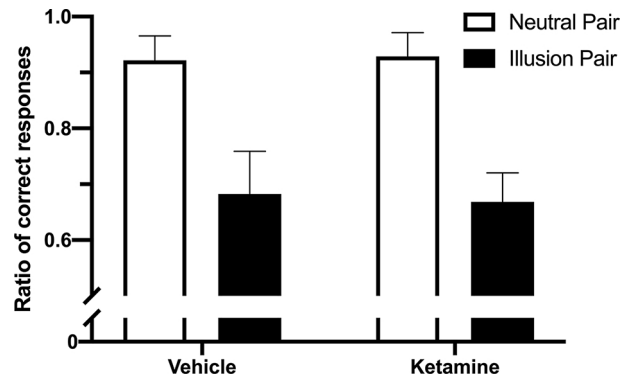

FIGURE 3 | Effects of ketamine administration on the percentage of correct responses between "illusion" and "neutral" pairs (mean + SEM; $n = 4$).

RESULTS

In Experiment 1, the PSE difference for the lines without arrowheads (10 ± 3.75 cm) was significantly smaller than those with arrowheads (40.4 ± 10.53 cm; $t_{(4)} = 6.175$; $p = 0.0035$; **Figure 2**). For the percentage of correct responses on the ketamine test, the repeated measures ANOVA indicated a significant difference between the illusion conditions ("neutral pairs" vs. "illusion pairs"; $F_{(1,12)} = 80.96$; $p < 0.0001$; **Figure 3**). The number of correct responses recorded for the "illusion pairs" was significantly lower than that for the "neutral pairs". On the other hand, there were no significant between-treatment differences ($F_{(1,12)} = 0.017$; $p = 0.898$) or factor interaction ($F_{(1,12)} = 0.138$; $p = 0.716$; **Figure 3**).

In Experiment 2, schizophrenic patients misplaced the center of the axis by a significantly larger margin than did the controls ($p < 0.05$; $t_{(14)} = 3.751$; **Figure 4**). The patients' estimations were off by 12.01 ± 0.72 mm (approx. 1.44° ± 0.08° visual angle), whereas the error margin for controls was 9.53 ± 0.49 mm

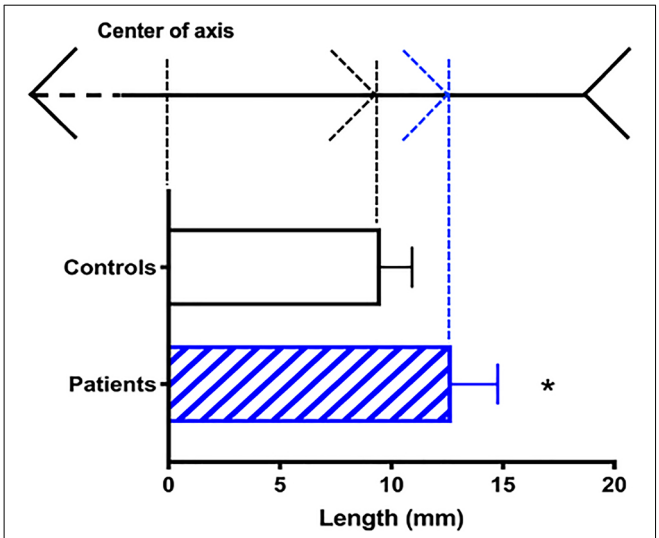

FIGURE 4 | Susceptibility to the Müller-Lyer Illusion among schizophrenic patients (blue) and controls (black). On the top: reference points in the illusion axis (not to scale). Average deviation in mm (mean + SEM) from the actual center of the axis ($n = 16$; $^*p < 0.05$).

(approx. 1.09° ± 0.06°; **Figure 4**). No correlation was found between MLI performance and PANSS main scores (positive: $p = 0.92$; negative: $p = 0.45$; cognitive: $p = 0.37$; data not shown).

DISCUSSION

The present study corroborated previous findings that capuchin monkeys are susceptible to the MLI and showed that chronic schizophrenic patients are more susceptible than controls. The effects of the MLI on the monkey's length judgment in Experiment 1 were similar to those reported in previous studies with capuchins (Suganuma et al., 2007; Jacobsen et al., 2017) and rhesus monkeys (Tudusciuc and Nieder, 2010). In the present experimental setup, the monkeys were not restrained during training or testing to reduce stress. This approach precluded the exact determination of the actual visual angle subtended by the stimuli on the screen. Nonetheless, given the average distance, the animals were from the screen during the task, we can estimate the judgment deviation (in this case, the difference between PSE and the actual length of the line) to be approximately 1.2°, similar to the values presented by Tudusciuc and Nieder (2010) in two rhesus monkeys.

In our experiment with the human participants, the task required sliding the central arrowhead in a Brentano version of the MLI. Interestingly, the average judgment deviation (i.e., the strength of the illusion) found in this modified task was that of 1.09° of the visual angle in healthy participants. This result is a little lower than that reported for the original version of the MLI in humans (1.19°: Tudusciuc and Nieder, 2010). The absence of time limits for completing each trial in our experiment may explain the difference across studies. In our task, participants took a variable length of time to slide the arrowhead, depending

on its starting position in each trial, but generally, it took longer than the general time limits used on forced alternative designs. As such, it has been reported that the strength of the Brentano may decrease if participants are allowed to inspect the stimulus (Predebon, 1998), but the duration of the inspection itself was not relevant. Despite this difference, the present task allowed for a faster and more precise (as confirmed by the low data dispersion in both groups) method to evaluate the judgment deviation in this task.

There has been some controversy regarding the sensitivity to MLI in schizophrenia patients. To our knowledge, the majority of reports have found robust changes compared to controls. These include increased susceptibility and/or impaired length estimation (Weckowicz and Witney, 1960; Capozzoli and Marsh, 1994; Rund et al., 1994; Parnas et al., 2001; Kantrowitz et al., 2009; Shoshina et al., 2011, 2014; Tolmacheva et al., 2018) or even reduced sensitivity depending on the progression of the disease (Parnas et al., 2001). Some reports, however, failed to find any significant differences (Tam et al., 1998; Grzeczkowski et al., 2018). Also, Kaliuzhna et al. (2019) argued that schizophrenia patients do not show perception abnormalities in early levels of visual hierarchy that supports a general impairment to visual illusions. Although, their results may corroborate the null findings for some visual illusions they did not test MLI specifically and, therefore, should not refute the several significant findings in the scientific literature on this illusion. The few conflicting results more likely stem from the methodological differences employed, as pointed out by King et al. (2017). Most studies used the standard illusion scheme with either a size-judgment or a forced-choice task which have been criticized in this context (Skottun and Skoyles, 2014). In this sense, the present approach, i.e., the sliding Brentano version, sought to provide a quantitative and sensitive measure of the participants' PSE which yields a high intra-subject reproducibility. We acknowledge that our results with patients are preliminary and based on a small sample size. It corroborates the results of most articles with MLI but care must be taken when comparing with other studies.

Another source of discrepancy among previous studies is sample heterogeneity and/or medication profile. Few studies have attempted to compare across schizophrenia subtypes/chronicity, let alone pharmacological treatment. One such study (Parnas et al., 2001) found that prodromal and first episode patients were less sensitive than controls in a forced-choice task with the Brentano version of MLI. On the other hand, chronic patients did not show a statistically higher susceptibility to the illusion. Here, the strength of the illusion was indeed higher for chronic patients compared to controls. The precision yielded by our sliding task may account for this difference, although Parnas et al. (2001) did not present a full statistical description in their results. Furthermore, we found that the MLI deviation was not correlated to the PANSS scores. Once again, this is hardly surprising given the low variability in the MLI results, in stark contrast to the psychometric 3-axis information evaluated by the PANSS scale. Alternatively, the lack of correlation might also reflect the absence of sensory/perception dimensions in the PANSS test. Therefore, it would be helpful to test a larger and more diverse sample of schizophrenia subtypes in the sliding Brentano task to characterize a possible range of illusion strength within this disorder and correlate it with the quantitative symptoms across the spectrum.

In a previous study, we found that NMDA receptor blockade by the antagonist MK-801 decreased the strength of the illusion in capuchin monkeys (Jacobsen et al., 2017). This effect seems to be in line with results from prodromal and first episode patients (Parnas et al., 2001). Here, we attempted an alternative strategy by blocking the receptor with ketamine. Surprisingly, the 4-day ketamine administration regimen failed to induce changes in performance as had been detected with MK-801. It is believed that NMDA blockage is the main pharmacological mechanism underlying the psychotic effects of ketamine and MK-801 (Lisman et al., 2008). Although both drugs share a common NMDA blocking site, ketamine shows a relatively lower affinity than MK-801 (Bresink et al., 1995). It also has a broader binding profile and is known to exert a wider range of effects, including anesthesia at higher doses. These effects may be induced by its moderate to strong interactions with cholinergic (Moaddel et al., 2013), dopaminergic (Kapur and Seeman, 2002), and opioid sites (Hirota et al., 1999), although the precise mechanisms are not well clarified.

One possible caveat to our results would be the relatively low dose chosen for ketamine. Previous studies with capuchin monkeys reported increase salivation, dystonia, reduced locomotor activity, and impaired reaction to stimuli in doses ranging from 2.5 to 5.0 mg/kg (Shigii and Casey, 1999, 2001). Taffe et al. (2002) found that 1.0 mg/kg, but not 0.3 mg/kg, of ketamine, disrupted cognitive performance in macaques. A prior pilot testing in our lab also showed mild ataxic movements at 1.0 mg/kg (data not shown). Since the present task required accurate precision for stimuli selection, we opted for a dose of 0.3 mg/kg. It is noteworthy that the dose used by Jacobsen et al. (2017) for MK-801 was also very low (5.6 μg/kg) and yet it was able to improve performance in this task. A low dose of ketamine also decreases the chance of a significant activation of aminergic or opioid systems. The discrepancies between ketamine and MK-801 effects in the MLI task are, therefore, more likely to stem from their different affinities with the NMDA receptor.

Regardless of the actual mechanism, currently available data seems to suggest that MK-801 may be the only effective drug to affect illusion strength in the present protocol. This may be somewhat problematic for this model since it restricts the range of effects that can be tested. Increasing MK-801 doses may easily induce ataxia which prevents MLI task execution. Therefore, even if higher doses could increase, rather than decrease, illusion strength, the current protocol would not be suitable to detect it. As sample sizes in monkey experiments are generally small due to ethical limitations, a continuous ketamine infusion protocol, similar to that used in human subjects (Lahti et al., 2001), may enable the individual

calibration of ketamine blood levels and ensure a stable, and possibly, a dose-dependent effect throughout the task duration.

Even though the sliding Brentano task may be too challenging to use with nonhuman primates, the forced-choice MLI task yields comparable results and may still be used for antipsychotic drug screening. It may also be tested in other models for schizophrenia, such as neonatal hippocampal lesion or PCP administration. On the other hand, given the range of sensory/perceptual alterations in patients and the lack of perceptual indices for diagnostical purposes, the task with Brentano is a promising tool in both research and clinical settings as it may be easily deployed on laptops or tablets for patient testing. In light of the results gathered so far on the MLI, further investigation is warranted on different schizophrenia subtypes and their immediate relatives, as well as a better refinement of nonhuman primate protocols.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Brasília Research Ethics Committee (CAAE 96510318.3.0000.0030). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Animal Ethics Committee of the University of Brasilia (46077/2014).

AUTHOR CONTRIBUTIONS

RM conceived the study, designed the experiment and wrote the first draft of the manuscript. RS was responsible for experimental setup, data collection, and analysis in Experiment 1. PC-C participated in experimental setup and data collection in Experiment 1. AC was responsible for experimental setup, data collection and analysis in Experiment 2. FS participated in experimental setup and data collection in Experiment 2. MB and FC participated in the experimental design planning, data analysis, and manuscript drafting. All authors contributed to the article and approved the submitted version.

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Rich Club Characteristics of Alcohol-Naïve Functional Brain Networks Predict Future Drinking Phenotypes in Rhesus Macaques

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Purpose: A fundamental question for Alcohol use disorder (AUD) is how and when naïve brain networks are reorganized in response to alcohol consumption. The current study aimed to determine the progression of alcohol's effect on functional brain networks during transition from the naïve state to chronic consumption.

Procedures: Resting-state brain networks of six female rhesus macaque (*Macaca mulatta)* monkeys were acquired using magnetoencephalography (MEG) prior to alcohol exposure and after free-access to alcohol using a well-established model of chronic heavy alcohol consumption. Functional brain network metrics were derived at each time point.

Results: The average connection frequency ($p < 0.024$) and membership of the Rich Club ($p < 0.022$) changed significantly over time. Metrics describing network topology remained relatively stable from baseline to free-access drinking. The minimum degree of the Rich Club prior to alcohol exposure was significantly predictive of future free-access drinking ($r = -0.88$, $p < 0.001$).

Conclusions: Results suggest naïve brain network characteristics may be used to predict future alcohol consumption, and that alcohol consumption alters functional brain networks, shifting hubs and Rich Club membership away from previous regions in a non-systematic manner. Further work to refine these relationships may lead to the identification of a high-risk drinking phenotype.

Keywords: magnetoencephalography, substance use disorder, risk factor, primate, brain function

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INTRODUCTION

Alcohol use disorder (AUD) constitutes a global problem and is ranked among the top substance abuse problems in the United States, with over 70% of adults that struggle with substance use disorder estimated to abuse alcohol (Substance Abuse and Mental Health Services Administration (SAMHSA), 2020). AUD impacts global brain functional

networks including the default mode, executive, attentional, salience and reward networks (Loeber et al., 2009; Park et al., 2010; Chanraud et al., 2011, 2013; Camchong et al., 2013; Sullivan et al., 2013) but the neurocircuitry underlying vulnerability and resilience to AUD is not clearly understood, making it difficult to establish viable, targeted treatment options.

This lack of clarity is due, in part, to the difficulty in capturing an alcohol-naïve baseline in human subjects. Clinical studies are often conducted with long-term drinkers at different drinking phases after changes in brain networks have already manifested. It is clear that AUD is characterized in part by dysfunctional information processing (Sullivan et al., 2000) that occurs in part through altered brain activity during both resting state (RS) and task performance in alcoholics (Rangaswamy and Porjesz, 2008) as compared to other neurological conditions (Georgopoulos et al., 2007). Functional brain networks including the default mode, salience, and executive networks are known to be sensitive to chronic alcohol use (Sullivan and Pfefferbaum, 2005; Sullivan et al., 2013; Weiland et al., 2014; Fede et al., 2019) however, the temporal nature and anatomic directionality of changes that occur remains unclear.

Studies such as IMAGEN have attempted to address this issue by evaluating adolescents prior to initiation of substance use (Maričić et al., 2020). Ivanov et al. (2020) used the IMAGEN study data to demonstrate that expected reductions in impulsivity were associated with lower levels of new onset drinking in an adolescent sample. They also found that blunted medial orbitofrontal activity in response to reward was associated with increased new onset and use. Nees et al. (2012) used the IMAGEN dataset to demonstrate that personality factors (novelty seeking, impulsivity, extraversion, and sensation seeking) were more strongly related to the initiation of drinking behaviors than reward related brain function, but that reward related brain function (particularly ventral striatum) was more strongly related to the development of problematic alcohol use. Heinrich et al. (2016) extended these findings in the IMAGEN study by including genetic factors. Results replicated the strong influence of personality factors on early initiation of drinking behaviors, while genetic factors appeared to become equally important when predicting future alcohol misuse behaviors. Reward related brain function did not contribute significant variance to the model. Similarly, Harper et al. (2019) used data from the Minnesota Twin Family Study Enrichment Sample to demonstrate that P3 amplitude mid-frontal theta power during an oddball task were predictors of new onset alcohol behaviors 3 years later in a sample of 14-year-old adolescents. While these studies offer valuable insight into the factors associated with initiation of use and onset of problematic use, they do not fully alleviate the confounding influence of environmental variables.

Non-human primate (NHP) models are valuable tools to help address the limitations of studies involving human participants (Grant et al., 2008). The model applied in the current study has been used to demonstrate that daily drinking for 15 months causes functional and genomic changes across the brain when contrasted against the alcohol naïve brain (Budygin et al., 2003; Floyd et al., 2004; Alexander et al., 2006; Carden et al., 2006; Anderson et al., 2007; Acosta et al., 2010; Cuzon Carlson et al., 2011; Mohr et al., 2013), as well as reorganization of brain networks measured by fMRI (Telesford et al., 2015) and significantly altered signal power of multiple bandwidths across the brain using magnetoencephalography (MEG; Rowland et al., 2017a). Using the same NHP model, alcohol naïve predictors of future drinking have also been identified. These include low cognitive flexibility (Shnitko et al., 2019), early drinking phenotypes (i.e., gulping vs. sipping; Grant et al., 2008; Baker et al., 2017), age, latency to begin drinking, and the number of "bouts" of drinking (Helms et al., 2014; Baker et al., 2017). No studies have examined alcohol naïve aspects of brain function as predictors of future drinking levels using this model.

The objective of the current study was a longitudinal examination of the trajectory of these changes at an earlier time point (6 months of chronic heavy alcohol intake) than previously examined (15 months chronic heavy alcohol intake) to identify at what point they begin to manifest and if baseline, alcohol-naïve indicators of future drinking can be identified.

MATERIALS AND METHODS

Animals

Adult female rhesus monkeys (n = 6, 5–7 years old at study start) were subjects in an ongoing ethanol (EtOH) self-administration study. This age group reflects late adolescence to early adulthood in humans. The monkeys were trained on an operant panel to self-administer all fluids and food using a well-established drinking model that parallels levels and patterns of intake observed in alcoholics (Grant et al., 2008; Baker et al., 2014). This process begins with EtOH-naïve monkeys that are induced to drink escalating doses of EtOH (0.5, 1.0 and 1.5 g/kg) for 30 days at each dose (induction phase). This phase introduces the monkeys to the reinforcing properties of alcohol and results in rapid and equal daily alcohol intake without causing taste aversion. All monkeys were maintained at 1.5 g/kg for 20 drinking days while operant panels were serviced and re-programed. Animals were then provided free access to EtOH and water for 22 h per day, 5 days per week for 180 days. Sessions began at 11:00 am each day. MEG recordings were acquired under EtOH naïve conditions (Baseline) and after 180 open access drinking days (Free Access) to determine the impact of chronic, daily intake on RS brain function. The alcohol-naïve baseline served as a within-animal control dataset.

Preparation for MEG Scans

Animals were fasted overnight from food but not EtOH prior to scans. Average time between last drink and sedation for imaging was 344.2 min (SD = 377.9, min = 0.0, max = 977) at the Free Access Scan. These time frames raise the possibility of acute withdrawal (Winger and Woods, 1973); however, symptoms of withdrawal were not observed during similar time frames on non-imaging days (Pieper and Skeen, 1977) and the anesthetic agent (propofol, a GABAA receptor positive allosteric modulator; Shin et al., 2018) helped ensure acute withdrawal symptoms were not present during data acquisition. Previous work has shown that acute withdrawal in this model peaks between 24–72 h (Cuzon Carlson et al., 2011), which

is beyond the duration since the last drink present here. Animals were sedated with ketamine (12 mg/kg, i.m.) for transport to the MEG suite. Anesthesia was induced with a bolus injection of 2.0–4.0 mg/kg propofol to allow intubation and was maintained *via* intravenous continuous infusion of 200 μg/kg/min propofol *via* syringe pump (Sage, Orion Research Corporation, Cambridge, MA, USA). Animals were placed in a supine position and artificially ventilated. These preparations are consistent with our previous reports (Telesford et al., 2015; Rowland et al., 2017a).

MEG Signal Recordings

Data were acquired using a whole head CTF Systems Inc. MEG 2005 neuromagnetometer system equipped with 275 first-order axial gradiometer coils. Head localization was achieved using a conventional three-point fiducial system (nasion and preauricular points). Each monkey was tattooed at each fiducial location to ensure consistent placement over time. Resting-state recording was conducted with animals lying supine for 5 min. Data were sampled at 1,200 or 2,400 Hz over a DC-300 or DC-600 Hz bandwidth, respectively. MEG data were preprocessed using synthetic 3rd order gradient balancing, whole trial DC offsetting, and band pass filtered from DC-80 Hz with powerline filtering. Data were visually inspected for obvious muscle artifact, and such epochs, if present, were discarded from further analyses. Following initial MEG recording, a T1 weighted MRI image was obtained for each animal for co-registration and localization of MEG signals.

Network Analysis

Network analysis was conducted identically to previous work (Rowland et al., 2017a,b, 2018). Network analysis proceeded by first identifying nodes of the network and quantifying communication among those nodes. The resulting matrices are conducive to the application of graph theory for calculating metrics describing the topology of the network.

Network Creation

Node Identification

For each animal 41 non-adjacent bilateral regions of interest (ROIs, voxel size = 2 × 2 × 2 mm) were identified in native brain space representing the default mode and reward networks. These networks have been previously demonstrated to be affected by chronic heavy alcohol consumption in humans (Chanraud et al., 2011; Müller-Oehring et al., 2015; Zhang and Volkow, 2019) and shown to be present in NHPs (Vincent et al., 2007; Mantini et al., 2011; Belcher et al., 2013). Brain regions included the anterior cingulate, medial and lateral orbital frontal cortex, principle sulcus, nucleus accumbens, caudate head and body, head of the putamen, parietal area, precuneus, lateral and medial amygdala, anterior, medial, and posterior hippocampus, vermis, anterior and posterior lobes of the cerebellum, thalamus, and anterior insula.

Functional Connectivity

Source series representing the unique weighted sum of the output across all MEG sensors for a specific ROI in the brain were calculated using a well-validated beamformer (synthetic aperture magnetometry, SAM; Robinson and Vrba, 1999; Hillebrand et al., 2005). Prior work has demonstrated the sensitivity of MEG beamformers to superficial as well as deeper sources (Stapleton-Kotloski et al., 2014, 2018). The weighted phase lag index (wPLI; Vinck et al., 2011) was calculated between all pairs of source series to establish functional connectivity, filtered between 1 and 80 Hz. The wPLI is a phase-based metric insensitive to fluctuations in source amplitude. A surrogate distribution of 5,000 unique pairs of phase-randomized time series was created for each animal individually (Prichard and Theiler, 1994). Connectivity was operationalized at the frequency with the greatest difference in wPLI value between the real and surrogate data, calculated as standard deviations. This approach allows the frequency at which connections occur to vary from connection to connection, representing a better model of brain activity than restricting connectivity to a specific frequency band (Chen et al., 2008; Hillebrand et al., 2012). To remove connections not different from noise, connections without a real-surrogate difference exceeding 2.5 standard deviations were left unconnected. The resulting networks were then thresholded by satisfying the equation $S = \log(N)/\log(K)$ where N represents the number of nodes in the network and K the average degree using $S = 2.5$ (Hayasaka and Laurienti, 2010).

Network Metrics

Network metrics calculated are listed in **Table 2**. Metrics were selected with a focus on characterizing the topology of the overall network. *Clustering Coefficient* was selected as an indicator of clustering and subgroup formation within the network. This metric was calculated as defined in Stam and Reijneveld (2007). *Modularity* was selected as an indicator of well-defined subnetworks within the larger network. This metric was calculated using the Louvain method of community detection (Blondel et al., 2008). The analysis was run 500 times, using the average number of modules (*Number Modules*) as outcome variables. *Assortativity coefficient* represents the correlation coefficient of the degree of nodes on each end of a connection. The degree of a node is the number of direct connections that node has to other nodes in the network. A positive coefficient suggests nodes are preferentially connecting to other nodes of similar degree, while a negative coefficient suggests nodes preferentially connect to those of different degree (Newman, 2002). Rich Club was selected as an indicator of the presence of a "network backbone." The Rich Club is a subset of highly connected and highly interconnected nodes forming the basis of the broader network. Rich Club characteristics (Colizza et al., 2006) were calculated using 500 independently generated random networks. The number of nodes (*Rich Club Nodes*) within the Rich Club, the minimum degree of those nodes (*Rich Club Degree*), and interconnectivity among those nodes (*Rich Club Coefficient*) were used as outcome variables. The Rich Club Coefficient was weighted by the average of the same metric across the 500 random networks, representing the level of increased interconnectivity over a random network. The *Mean Connection Frequency* was calculated as the average of the frequency at which connections occurred across all connections in the network. The number of connections occurring in each of the canonical

frequency bands (e.g., delta, theta, alpha, beta, gamma) was also calculated as outcome variables. *Hubs* of the network were identified as the 10% of nodes ($n = 4$) with the highest degree.

Materials

Beamforming and source series construction were completed using software provided by CTF MEG International Services LP (Coquitlam, BC, Canada). Further analyses of source series data and network creation were conducted using Matlab 2016a. Network metrics were calculated using the Brain Connectivity Toolbox (Rubinov and Sporns, 2010). SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Analyses

Differences across time in network metrics (Baseline to Free Access) were examined using paired samples *t*-tests. Consistency over time in the distribution of hub and Rich Club members was examined using paired samples *t*-tests. The data used are presented in **Tables 4**, **5**. The independent variable was time point, and the dependent variable was the distribution of membership across regions. Secondary Chi-Square analyses (McNemara's test) were run to examine how the hub and Rich Club status of individual brain regions changed over time. Spearman rank correlations were conducted to examine the relationship between network metrics and drinking outcomes (daily average g/kg) during Free Access. Two-tailed tests and alpha of 0.05 were used for significance.

RESULTS

Daily average g/kg EtOH consumption increased significantly once given free access [mean (SD); Free-Access = 4.7 (1.0), $p < 0.01$, Cohen's $d = 5.23$], as shown in **Table 1**. Network metrics at Baseline and Free-Access (after 180 days unrestricted access) are shown in **Table 2**.

Networks Predicting EtOH Consumption

Table 3 illustrates correlations between Baseline (EtOH naïve) network metrics and Free Access consumption. The minimum degree of the Rich Club at baseline was strongly related to Free Access consumption levels (**Figure 1**).

Effects of EtOH on Networks

Table 2 shows mean and standard deviations of network metrics at Baseline and Free Access. The average connection frequency was significantly higher at Free Access than at Baseline, $t_{(5)} = -3.20$, $p = 0.024$, but no other differences in network metrics were observed.

Specific Brain Regions

Table 4 demonstrates areas considered hubs across animals at each time point. For brain structures with multiple aspects, the region was considered a hub if any of the aspects were considered a hub (e.g., if either the posterior, medial, or anterior hippocampus was a hub, then **Table 4** indicates the hippocampus as a hub). There was no significant change in brain regions considered hubs from Baseline to Free Access. **Table 5** includes brain regions that were members of the Rich Club for at least three animals at any time point, again collapsing within regions. A significant decrease in the commonality of regions in the Rich Club across animals was observed from Baseline to Free Access, $t_{(9)} = 2.74$, $p = 0.022$. Tests of change in individual regions were not significant.

TABLE 1 | Amount of EtOH consumed by subjects during the Free-Access Period in grams/kilogram.

Subject	Free access
1	3.8
2	4.4
3	5.4
4	5.5
5	3.3
6	5.9

Note. n = 6. EtOH = ethanol.

TABLE 2 | Descriptive statistics of network metrics prior to and following exposure to ethanol.

Network metric	Baseline	Free access
Clustering coefficient	0.35 (0.1)	0.30 (0.1)
Global efficiency	0.59 (0.05)	0.56 (0.05)
Assortativity coefficient	−0.40 (0.1)	−0.19 (0.2)
Rich club coefficient	1.84 (0.1)	1.91 (0.3)
Rich club nodes	13.17 (1.7)	10.83 (4.3)
Rich club minimum degree	7.50 (1.1)	9.50 (2.7)
Number of modules	10.50 (3.4)	8.83 (4.4)
Mean connection frequency[a]	6.57 (5.7)	16.01 (10.6)
Delta connections	172.67 (96.0)	99.0 (99.7)
Theta connections	15.33 (25.4)	34.67 (45.4)
Alpha connections	4.00 (9.8)	15.33 (23.2)
Beta connections	68.33 (106.9)	58.33 (47.9)
Gamma connections	1.67 (4.1)	54.67 (64.5)

Data are presented as mean (standard deviation). Note. n = 6. [a]Paired samples t-test between EtOH Naïve Baseline and Free-Access drinking $p < 0.05$; EtOH = ethanol.

DISCUSSION

The current study demonstrates that characteristics of the Rich Club of alcohol-naïve brain networks are related to future drinking behaviors. In addition, following extended chronic drinking the connection strength in the network was altered; however, no effect on network metrics was observed. Finally, while hubs of the network were not observed to change significantly over time, membership in the Rich Club was significantly altered by chronic heavy drinking.

Aspects of *alcohol-naïve* resting-state functional brain networks were demonstrated to predict *future* drinking levels. Higher minimum degree of the Rich Club at Baseline (alcohol-naïve) was strongly correlated with decreased alcohol consumption during the future free access period. The Rich Club is a community of nodes within the network that have high degree and interconnectedness, serving as the "spine" of the network (Colizza et al., 2006). These nodes represent hubs within the network and communication among these nodes can often serve as "shortcuts" within the network, increasing efficiency of communication across otherwise distantly connected nodes. As the minimum degree of the

TABLE 3 | Correlations between Baseline network metrics and Free-Access drinking levels.

	Connection frequency	Rich club coefficient	Rich club nodes	Rich club degree	Clustering coefficient	Assortativity
Free Access drinking	0.60	−0.26	0.71	−0.88[a]	−0.31	0.03

Note. n = 6, Free-Access Drinking = average daily consumption in grams/kilogram during the 180 days of unrestricted access, [a]p = 0.02.

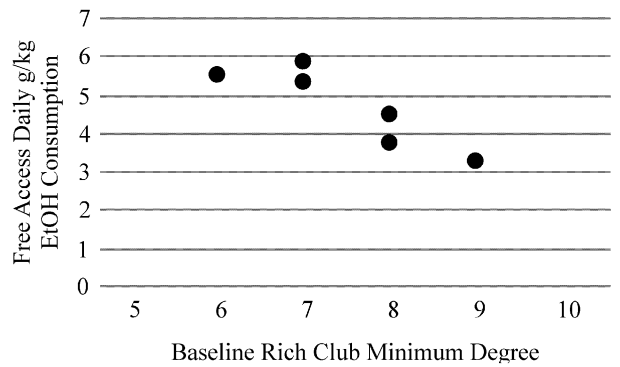

FIGURE 1 | The minimum degree of the Rich Club of the alcohol naïve (Baseline) functional brain network was strongly related to future drinking when animals were provided free-access to alcohol ($r = -0.88$, $p = 0.02$).

TABLE 4 | The number of subjects for which each brain region was considered a hub of the network.

Brain region	Baseline	Free-Access
Parietal	3	0
Thalamus	4	2
Precuneus	2	1
Cerebellum	4	2
Amygdala	2	2
Hippocampus	2	4

Nodes are considered hubs if they are in the upper 10% of the network for degree. Only nodes considered a hub for at least two subjects at any time point are presented. Note. n = 6.

TABLE 5 | The number of subjects for which each brain region was considered a member of the Rich Club.

Brain region	Baseline	Free-Access
Putamen	5	4
Hippocampus	5	5
Thalamus	5	3
Insula	5	2
Cerebellum	5	3
Parietal	4	3
Amygdala	4	5
OrbitoFrontal	4	3
Caudate	3	3
Precuneus	3	2

Note. n = 6.

Rich Club decreases, the centrality of Rich Club nodes also decreases, meaning fewer aspects of communication are routed through these nodes. Essentially, as the minimum degree decreases, the Rich Club becomes less differentiated from the other nodes in the network. This result suggests that the level of Rich Club differentiation may be predictive of future drinking levels, *even when measured prior to alcohol exposure*.

Alterations to the Rich Club subnetwork are likely to have broad and sweeping effects on brain communication and information processing (van den Heuvel and Sporns, 2013). Differences in Rich Club characteristics have been observed in many neurodevelopmental disorders, including schizophrenia (Collin et al., 2017), bipolar disorder (Wang et al., 2019), and autism (Hong et al., 2019). As such, the broad differences in Rich Club characteristics observed in this study are unlikely to serve as a direct "neurophenotype" of AUD without further refinement and empirical study. However, these results identify that differences in network topology are important to understanding individuals who might be at risk for future heavy drinking or AUD. Further, these results are consistent with previous work using the same NHP model indicating that premorbid behaviors and those occurring early in the drinking history may be predictive of future consumption levels. These factors include low cognitive flexibility (Shnitko et al., 2019), early drinking phenotypes (i.e., gulping vs. sipping; Grant et al., 2008; Baker et al., 2017), age, latency to begin drinking, and the number of "bouts" of drinking (Helms et al., 2014; Baker et al., 2017). The current results are the first to provide a brain-based factor indicative of future drinking in this model, suggesting that alcohol-naïve differences in Rich Club characteristics of functional brain networks also predict future drinking in this model.

These results extend recent findings in human participants demonstrating that white matter brain networks of individuals with AUD displayed lower Rich Club characteristics compared to their non-abusing siblings, who displayed lower levels compared to control participants (Zorlu et al., 2019). These results are also consistent with recent findings using the same NHP model indicating that chronic heavy drinking inhibits white matter growth (i.e., reduced connectivity) during late adolescence (Shnitko et al., 2019). While Zorlu et al. (2019) suggest potential premorbid differences in white matter network structure may be a marker of risk or susceptibility to AUD, the results of the current study provide direct empirical support for this hypothesis, showing that Rich Club characteristics of premorbid functional brain networks are directly related to future drinking levels. These results are also consistent with those of several longitudinal projects conducted in humans including the IMAGEN and NCANDA studies (Brumback et al., 2016; Maričić et al., 2020; Silveira et al., 2020). However, the current results examine functional RS brain networks and not reward based processing or structural aspects. The current study also did not acquire genetic or behavioral data as was acquired by IMAGEN, limiting further comparisons. Findings from the current study as well as these previous studies demonstrate that

brain function and structure measured prior to exposure to alcohol may be able to identify individuals at risk for future heavy alcohol consumption.

Alcohol-induced changes in functional brain networks were observed following a period of exposure and free access to alcohol. The mean connection frequency increased following chronic heavy drinking; however, changes in network metrics were not observed. This suggests the general topology of networks were not altered (e.g., path lengths, clustering, etc.) Significant changes were seen in the membership of the Rich Club. There was much less consistency in Rich Club membership following chronic heavy drinking, suggesting the networks were being altered in an inconsistent manner across animals. It should be noted that the quantity of alcohol consumed during the free access period was fully determined by each animal. These results support the potential for a dose-dependent relationship between patterns of alcohol consumption and the effect on functional brain networks (Correas et al., 2015, 2016; López-Caneda et al., 2017; Perez-Ramirez et al., 2017).

Limitations of the current pilot study include the small sample size, which limits the complexity and sensitivity of analyses that can be conducted. Neuroimaging was conducted under anesthesia, which has known effects on brain function (Boveroux et al., 2010; Xie et al., 2013; Guldenmund et al., 2016). Possible interactions between the anesthetic and alcohol could have occurred, if not directly, then through the indirect development of tolerance. However, anesthesia was maintained at consistent levels and physiological indicators of arousal were monitored continuously, suggesting that levels of sedation were consistent across scans and animals. Additionally, after baseline data was acquired in the alcohol-naïve state, all animals entered the alcohol self-administration paradigm. We are mindful that this within-animal comparison across time limits insight into potential non-alcohol related changes over time or changes as a result of operant manipulations. However, test-retest scan sessions in monkeys 1 year apart and in humans 6 years apart (Stapleton-Kotloski et al., 2014) have established that networks remain stable over time.

Neuroimaging under conscious conditions will be required to completely understand the effects of alcohol on brain function using this model. The interval between ethanol access and MEG scans raises the possibility that some animals may have been experiencing symptoms of withdrawal (Winger and Woods, 1973). However, signs of withdrawal were not observed during the same time periods on non-imaging days (Pieper and Skeen, 1977; Cuzon Carlson et al., 2011). Also, propofol was used as the anesthetic agent, helping to ensure animals were not experiencing withdrawal symptoms *during* scans (Shin et al., 2018). Finally, animals who ceased alcohol consumption prior to scans did so voluntarily and in a time frame consistent with non-imaging days and were not forcibly fasted.

CONCLUSIONS

The current study identified a relationship between functional brain networks in the alcohol-naïve state and future alcohol consumption, consistent with other work using this model demonstrating early behavioral markers of future drinking. This is the first brain-based predictor of future alcohol consumption identified for this model. Additionally, significant alteration in the Rich Club of the network was observed following 180 days of chronic heavy consumption of alcohol, an earlier time point than examined in previous works. Future work will be invaluable in clarifying the changes, and specifying the timing of those changes, that infer risk specific to AUD in humans.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Wake Forest School of Medicine ACUC.

AUTHOR CONTRIBUTIONS

JR, JS-K, AD, DG, and JD contributed to the study design, JR, JS-K, AD, GA, PE, and JD contributed to data acquisition. JR, JS-K, DG, GA, and JD contributed to data analysis and interpretation. JR, JS-K, GA, AD, PE, DG, and JD contributed to manuscript preparation. All authors contributed to the article and approved the submitted version.

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Natural and Experimental Evidence Drives Marmosets for Research on Psychiatric Disorders Related to Stress

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Knowledge of the behavioral ecology of marmosets carried out in their natural habitat associated with the advent of a non-invasive technique for measuring steroid hormones in feces has made a significant contribution to understanding their social relationships and sexual strategies. These studies showed that they are mainly monogamous, live in relatively stable social groups according to a social hierarchy in which females compete and males cooperate, and form social bonds similar to humans, which makes this species a potential animal model to study disorders related to social stress. In addition, laboratory studies observed the expression of behaviors similar to those in nature and deepened the descriptions of their social and reproductive strategies. They also characterized their responses to the challenge using behavioral, cognitive, physiological, and genetic approaches that were sexually dimorphic and influenced by age and social context. These findings, added to some advantages which indicate good adaptation to captivity and the benefits of the birth of twins, small size, and life cycle in comparison to primates of the Old World, led to their use as animal models for validating psychiatric diseases such as major depression. Juvenile marmosets have recently been used to develop a depression model and to test a psychedelic brew called Ayahuasca from the Amazon rainforest as an alternative treatment for major depression, for which positive results have been found which encourage further studies in adolescents. Therefore, we will review the experimental evidence obtained so far and discuss the extension of the marmoset as an animal model for depression.

Keywords: New World primates, behavior, HPA axis, animal model, depression

INTRODUCTION

Several species and numerous protocols have been tested to validate translational animal models which better mimic the physiological and behavioral states observed in human psychopathologies associated with chronic social stress including fish species (Øverli et al., 2004; Schjolden et al., 2005), birds (Roberts et al., 2007), rodents (Sgoifo et al., 1999; Veenema et al., 2003; Kompagne et al., 2008; Martinez et al., 2008; Hennessy et al., 2009), sheep (Stackpole et al., 2006), pigs (van der Staay et al., 2008), and non-human primates (Barros and Tomaz, 2002; Dettling et al., 2007).

These models show mammals present great genetic similarities and share corresponding neurophysiological mechanisms involved in socialization. However, their social organizations reveal a large range of differences in their size, composition, social bonding, parental care, and reproductive skew, creating a diversity of societies which is currently under investigation in terms of studying their complexity. This evidence demonstrates that the evolutionary pressures related to the natural environment demand adaptive responses which lead to a great diversity of strategies, thus making it difficult to apply the findings of some animal models to people. Therefore, these interspecific differences limit the suitability of a given species as an experimental model for comparative studies with humans (Kappeler, 2019), including non-human primates.

The common marmoset is a small New World primate and has gained attention mainly due to its complex social organization. This species has several social behaviors which are linked to auditory and visual cues like humans, including that they show gazing behavior, intentional pro-social behavior (Burkart and van Schaik, 2020; also known as altruism in humans), and observational social learning. In fact, their social cognition and communication seem more similar to that of humans than those of rhesus monkeys (Burkart and Finkenwirth, 2015). It is also suggested that humans and marmosets share main traits as cooperative breeders such as social tolerance and social monitoring resulting from cooperative breeding (Burkart and Finkenwirth, 2015).

Marmosets exhibit a range of changes at physiological and behavioral levels when facing stressful situations which depends on the individual, social and environmental characteristics resembling those observed in humans. They also have a well-defined ethogram (Stevenson and Poole, 1976), and there are procedures to measure cortisol in blood and urine, a non-invasive technique using feces (see de Sousa, 2017), and new methods have more recently been standardized for saliva (Cross and Rogers, 2004; Cross et al., 2004; Ash et al., 2018) in the laboratory and in hair in captive (Phillips et al., 2018) and wild marmosets at different ages (Garber et al., 2020).

These features make this species relevant for their use as a model in psychiatry studies related to chronic stress. It is largely understood that chronic social stress is the main etiology of Major Depressive Disorder (MDD; Tafet and Bernardini, 2003), General Anxiety Disorder (GAD; Juruena et al., 2020), and Post-Traumatic Stress Disorder (PTSD; Yehuda et al., 2015). In this sense, the cortisol profile is considered the marker of hypothalamus-pituitary-adrenal (HPA) axis disruption and has been used as a potential biomarker of some psychiatric syndromes (Dieleman et al., 2015; Herman et al., 2016). Thus, in this minireview, we aim to analyze recent studies to provide more evidence for the use of common marmosets as one of the strongest candidates today as an experimental model for research on psychiatric disorders related to stress.

BEHAVIORAL AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS CHARACTERIZATION IN COMMON MARMOSETS

Despite there being several protocols for inducing psychiatric disorders in animal models such as genetic (Sasaki, 2015), pharmacological (Costall et al., 2011; Pryce et al., 2011; Yasue et al., 2015), and behavioral (Cilia and Piper, 1997; Barros et al., 2000, 2008; Dettling et al., 2002b), the etiological models for mental disorders associated to chronic stress based on social challenging are closer to that which triggers human PTSD (Dettling et al., 2002a,b, 2007), GAD (Cilia and Piper, 1997; Barros et al., 2000, 2008) and MDD (Johnson et al., 1996; Galvão-Coelho et al., 2008, 2017). Therefore, animal models are promising to gain larger emphasis in scientific studies. In order to do so, it is essential to investigate and understand the neurobiological mechanisms underlying the associated physiological and psychological mechanisms that support the analysis of psychiatric disorders. The data obtained in studies using non-human models have significant limitations because the etiology of psychiatric disorders is characterized by a multiplicity of causal factors and symptoms (Salgado and Sandner, 2013). Furthermore, in the perspective that it is the combination of "nature" and "nurture" which contribute to different phenotypes of susceptibility and resilience to developing mood disorders (Hollander et al., 2020), the need for substantial information to attain the validation criteria of an animal model is strengthened.

The systematic study of the behavior and development of common marmosets (*Callithrix jacchus*) living in captivity in colonies worldwide started in the 1970s (Epple, 1970; Abbott and Hearn, 1978), and wild groups started to be monitored in Brazil in the 1980s (Stevenson and Rylands, 1988). In this context, the common marmoset is currently one of the most studied species among non-human primates in both wild and in captivity situations and consequently, the marmoset emerges as a distinct experimental model with respect to the amount of information available in both settings.

Natural Environmental Studies

Common marmosets are small New World primates of the Callitrichidae family. This species is not threatened and easily found in Brazilian Atlantic Forest, usually living in stable social groups composed from three to 19 familiar individuals (Stevenson and Rylands, 1988; Digby, 1995; Schiel and Souto, 2017). The group composition varies depending

on the number of births, migrations, and disappearances (Digby and Barreto, 1993). Studies also show that they are cooperative breeders, suggesting extended family groups in which reproductive pairs (dominants) develop special social bonds, present parental and alloparental care more frequently involving older siblings and close relatives (Digby, 1995). Despite groups generally being composed of relatives, unrelated members have also been identified in the social group (Faulkes et al., 2009).

The core of the social group is the monogamous breeding couple, which has very low sexual body dimorphism, but polygynous groups have also been described (Digby, 1999; Arruda et al., 2005). It is interesting to note that marmoset males are more cooperative and build stronger intra-sexual ties, while female marmosets exhibit higher intra-sexual competition levels (Arruda et al., 2005; Yamamoto et al., 2010; de Sousa et al., 2019).

The intra-group organization of marmosets can be disturbed during social reproduction dynamics, as shown in situations where dominant females perpetrated infanticide toward the offspring of subordinates (Digby, 1995; Arruda et al., 2005) or during the subordinate scaping of ovulatory inhibition (Albuquerque et al., 2001), as well as when males were facing territorial dispute challenges (Digby, 1995; Lazaro-Perea, 2001), thus producing tensions and activating the stress response system (de Sousa et al., 2019). Furthermore, both behavioral repertoire (episodes of aggression and open fights expressed by piloerection displays, scent marking, and anogenital presentation) and associated endocrine correlates increased cortisol in females and cortisol and androgens in males during these situations, being characteristic of the stress response of the animals when exposed to natural stressors. These reactions characterize the typical stress response that is shared by mammals, including humans.

Laboratory Studies

The first studies developed in common marmosets under captive conditions date from the 1970s throughout the 1980s and described data related to marmosets' behavior living in colonies, such as: living in family groups (Epple, 1970), breeding and development (Abbott and Hearn, 1978; Tardif et al., 2003), parental care (Ingram, 1977), first cohabitation weeks of common marmoset heterosexual pairs (Evans and Poole, 1983; Woodcock, 1983), and the construction of an ethogram of social behavior in different contexts (piloerection displays, adult play, copulatory, aggressive, and prey-catching behavior and vocalization; Stevenson and Poole, 1976). These studies were fundamental to provide information to researchers to learn about and better understand the role of their behaviors and how they vary along with their age, as well as the endocrine profile in the transition phases of puberty (Abbott and Hearn, 1978), first ovulation and initial age for pregnancy (Tardif et al., 2003). Three classifications were proposed to characterize the development stages of the common marmoset (Missler et al., 1992; Yamamoto, 1993; de Castro Leão et al., 2009), and a comparative analysis was carried out (Schultz-Darken et al., 2016) providing a reference scale for long-term studies.

A longitudinal study by our laboratory on the developmental stages of common marmosets from the infant stage (3 months) to the sub-adult (15 months) describes the cortisol variation in males and females in relation to age and sex. Cortisol changes throughout the marmoset's life stages were recorded, with the highest cortisol levels being observed in the infant phase and tending to decrease with development, especially in males (de Sousa et al., 2015). A better understanding of marmoset development allows appropriate parameters to be available to compare the normal and abnormal development after birth and to assist in psychiatric studies of early life chronic stress (Dettling et al., 2002a,b, 2007; Pryce et al., 2004a,b, 2011; Arabadzisz et al., 2010) and to assess neurodevelopment from the perspective of using biomedical research in transgenic approaches (Sasaki et al., 2009).

Sexual dimorphism is observed from the juvenile stage at baseline cortisol levels in favor of female marmosets, which have higher fecal cortisol levels than males (de Sousa et al., 2015). However, despite the sex, it is suggested that the marmoset's temperament also modulates basal cortisol levels, with three distinct basal cortisol profiles being described in adult marmosets of both sexes scaled as low, medium, and high (Galvão-Coelho et al., 2008).

Moreover, a sexual dimorphic stress response was evidenced by distinct cortisol increases when adult marmosets were moved to new cages, which varies depending on the type of stressor, the presence or not of a co-specific, and the type of social support (Galvão-Coelho et al., 2012; de Menezes Galvão et al., 2016). Dyad formation also triggered a sexual dimorphic stress response in adult marmosets. Females were more reactive than males when facing a pair formation as well as in response to isosexual dyad formation, independent of the co-specific being familiar or not (Galvão-Coelho et al., 2012). Adult male marmosets were also more sensitive to social isolation, showing larger cortisol increases (Castro and de Sousa, 2005).

Other convergent data in terms of sexual dimorphism in the stress response in marmosets was evidenced in a genetic study where the NTRK2, CREB1, and CRTC1 genes possibly associated with CREB1-BDNF-NTRK2 pathway have a higher expression level in females, suggesting greater demand for this signaling cascade in females (Nogueira et al., 2018). This pathway presents an important role in brain adaptation to stress and variations in these genes have been identified as probable risk factors for depression in humans (Juhasz et al., 2011).

Stress and psychiatric studies are usually based on male models. However, there is a long-standing demand for psychiatric models which include both sexes (Kokras and Dalla, 2014; Alshammari, 2021). Therefore, sexual dimorphism in the HPA axis and in the autonomic component of the stress response in marmosets are important characteristics of this species which resemble humans and enables its use in sex-related psychopathology studies. It is assumed that better treatments can be validated if both sexes are included as pre-clinical models of psychiatric diseases (Dalla et al., 2010).

Laboratory studies also demonstrated a diurnal (de Sousa and Ziegler, 1998; Ferreira Raminelli et al., 2001) and

annual fluctuation in plasma cortisol in common marmosets over a 2-year interval in which a marginal effect of sex was observed with females showing higher levels during dry seasons (Cunha et al., 2007). The annual variation in cortisol was associated with precipitation and temperature, but not with the photoperiod, due to the small variation in the photophase length at the Equator line where our animals live. Therefore, the circadian and seasonal change in cortisol can also contribute to approaches which explore some characteristics in physiological regulation using appropriate protocols which may be relevant for models to investigate circadian changes in the cortisol profile of depressed patients (Keller et al., 2006) and for seasonal depression (Thalén et al., 1997).

THE COMMON MARMOSET AS AN ANIMAL MODEL TO STUDY PSYCHOPATHOLOGIES ASSOCIATED WITH CHRONIC STRESS

Despite recognition of increasing psychiatric disorder incidences being related to chronic stress in adolescents and the importance of chronic stress in this ontogenetic phase on long–term non-adaptive neuroplasticity changes (Thapar et al., 2012; Knowles et al., 2021), a large part of the literature about chronic stress related to neuropsychiatric disorders using marmoset models have only explored adult (Johnson et al., 1996; Cilia and Piper, 1997; Barros et al., 2000, 2008) and infant (Dettling et al., 2002a,b, 2007) ages. Therefore, being aware of this gap, our group recently validated the marmoset as a juvenile animal model for major depression (Galvão-Coelho et al., 2017).

In aiming at the complete validation of this juvenile marmoset animal model for major depression, we developed a protocol addressing: (a) ecological validity, which means that the symptoms in this model were triggered by similar occurrences for human diseases, and in this case we used the social isolation protocol; (b) construct validity: animals showed activation of physiological processes in a similar way to humans, such as cortisol levels; (c) face validity: the animals expressed symptoms/behavior changes similar to those of humans, such as changes in appetite and anhedonia; (d) predictive validity: animals and humans showed similar responses to treatments; (e) interrelational validity: we investigated the model in several domains such as behaviors, molecular biomarkers and cognition; (f) evolutionary criteria: reflects the ability of the proposed model to investigate specific domains in various species such as anhedonia, body weight and cortisol analysis, which can be done on marmosets, humans and other species such as rodents; and (g) population validity: the capacity of the proposed model to reflect the natural variance of phenotypes observed in the general population, as observed in some studies in the literature (Galvão-Coelho et al., 2008, 2017).

In this context, male and female juvenile marmosets (approximately 7 months) were subjected to a chronic social stressor protocol (13 weeks of social isolation) to induce a state of depression. Next, fecal cortisol, body weight, and some intra and interspecific behaviors were analyzed in the stressed group and compared with a control group where marmosets continued to live with the family throughout the study. In short, we observed a behavioral and neuroendocrine depressive state similar to patients with depression (Dean and Keshavan, 2017; Santiago et al., 2020) and other non-human primate models of depression (Li et al., 2013; Hennessy et al., 2014) for both sexes isolated from marmosets (male; female), but not for animals kept with the family. Symptoms included a significant reduction in cortisol levels, an increase in anxiety-like behavior (stereotypes, such as scratching and body piloerection), presence of anhedonia, sexual dimorphic changes in diet and body weight (Galvão-Coelho et al., 2017). Most of the altered behaviors, such as scratching, as well as the change in cortisol levels, were reversed by treatment with nortriptyline (7 days), but not by the vehicle (Galvão-Coelho et al., 2017).

The validation of this protocol gave way to developing depressive juvenile models with other non-human primate species (Teng et al., 2021) and enabled a step forward when a potential new alternative drug therapy (nortriptyline) for depression was tested (da Silva et al., 2019). Using that same validated protocol, some male and female juvenile marmosets (male; female) were treated with a single dose of ayahuasca (da Silva et al., 2019). Ayahuasca is a psychedelic decoction made from combining two plants from the Amazon rainforest: *Psychotria viridis* and *Banisteriopsis caapi*, which has shown promising fast antidepressant effects in humans (Palhano-Fontes et al., 2014, 2021; Perkins et al., 2021; Sarris et al., 2021). Thus, we observed a fast recovery in the decreased cortisol levels (hypocortisolemic state) and the bodyweight of depressive-like marmosets to the baseline values 24 h after an acute dose of Ayahuasca, and not after the vehicle, as well as reductions in anxiety-like stereotypic behaviors. Additionally, the single dose of ayahuasca also had long-lasting effects (14 days) and seemed to be more promising than nortriptyline treatment (da Silva et al., 2019).

These findings in the marmoset supported the development of clinical trials using ayahuasca treatment for resistant depression and showed positive results, including some similar to those seen in the marmoset (for example, regulating cortisol levels; de Almeida et al., 2019; Palhano-Fontes et al., 2019; Galvão-Coelho et al., 2020, 2021).

FUTURE DIRECTIONS

As mentioned earlier in this review, some scientists argue that current animal models are insufficient to express the full extent of mood disorders and therefore have no real value in understanding mental illness. However, new research initiatives to integrate the effort to study mental disorders, such as the Roadmap for Mental Health Research in Europe (ROAMER; Haro et al., 2014) and Research Domain Criteria (RDoC), funded by European and American public and private institutions (Insel et al., 2010; Elfeddali et al., 2014) intend to build a matrix of variables which constitute a unit of analysis involving studies from genetic, physiological, behavioral, and self-report evaluations. New information is added or contributions are

made in these projects by revision of earlier knowledge on psychopathology analysis of symptoms, and both initiatives will facilitate progress in this field. They argue that these disorders are too complex to try to understand globally, so the knowledge gained step-by-step should provide more clarity and effectiveness. In addition, some specific symptoms are common to different psychopathologies, so that understanding one symptom for a certain disorder can be applied to another. Therefore, this new understanding makes studies on animal models even more important, since it is easier to focus on fewer symptoms and their pathophysiological correlates and their management. In turn, encouraging the development of new protocols and stimulating new studies on biomarkers, as well as on the development of new drugs. In addition, marmosets are the focus of a large–scale Japanese project called Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS; Okano and Mitra, 2015; Okano et al., 2015), and new colonies of marmosets show that the use of this small neotropical primate as experimental models continues to present a promising future for scientific research, including for investigations of neuropsychiatric diseases.

AUTHOR CONTRIBUTIONS

MS and NG-C wrote and edited the manuscript. MS, NG-C, and MM performed bibliographic research and main drafting. All authors contributed to the article and approved the submitted version.

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REVIEW
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A Rhesus Monkey Model of Non-suicidal Self-Injury

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Non-suicidal self-injury (NSSI) is a type of behavioral pathology seen not only in a variety of clinical conditions but also among non-clinical populations, particularly adolescents and young adults. With the exception of rare genetic conditions that give rise to self-harming behaviors, the etiology of NSSI and the events that trigger specific episodes of this behavior remain poorly understood. This review presents the features of an important, extensively studied animal model of NSSI, namely spontaneously occurring self-injurious behavior (SIB) in rhesus macaque monkeys. We compare and contrast rhesus monkey SIB with NSSI with respect to form, prevalence rates, environmental and biological risk factors, behavioral correlates, proposed functions, and treatment modalities. Many parallels between rhesus monkey SIB and NSSI are demonstrated, which supports the validity of this animal model across several domains. Determining the etiology of spontaneously occurring SIB in monkeys, its underlying biological mechanisms, and which pharmacological agents are most effective for treating the disorder may aid in identifying potential risk factors for the occurrence of NSSI in humans and developing medications for severe cases that are resistant to conventional psychotherapeutic approaches.

Keywords: self-injurious behavior, non-suicidal self-injury, rhesus monkey, hypothalamic-pituitary-adrenocortical axis, early life stress, opioid system, sleep disruption

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INTRODUCTION

The objective of this review is to examine the applicability of a rhesus monkey model of self-injurious behavior (SIB) for non-suicidal self-injury (NSSI) in humans. Other models based on pharmacological or genetic manipulations of laboratory rats and mice have been instrumental in studying NSSI associated with genetic syndromes such as Prader-Willi and Lesch-Nyhan, as well as neurodevelopmental disorders like autism (Knapp and Breese, 2016; Devine, 2019). Indeed, these models have pointed to a possible role for the dopamine system in mediating the self-injury observed in those syndromes and disorders. In contrast, the animal model reviewed here differs from experimental rodent models in three important ways: (1) the model is based on a higher primate species that possesses communicative, social, and cognitive abilities more similar to humans than those of rodents; (2) SIB in our model arises spontaneously, without the need for pharmacological treatments; and (3) the model is particularly directed toward understanding NSSI that occurs in the general population and in certain psychiatric disorders such as posttraumatic stress disorder.

The rationale for evaluating the rhesus monkey model of SIB is based, in part, on disturbing trends showing that NSSI is on the rise among teenagers and young adults (Wester et al., 2018)

and on significant animal welfare concerns for non-human primates with this condition (Novak, 2020). Humans and Old World monkeys such as rhesus macaques share many important features including a long lifespan, complex social structures, higher order cognitive processes, large brains in relation to body size, and hands adapted for manipulation of objects. From infancy to adulthood, monkeys pass through key developmental stages similar to those in humans. Additionally, Old World monkey brains contain all the major prefrontal cortical subdivisions seen in the human brain (Carmichael and Price, 1994). These similarities suggest that animal models based on these species can serve as an excellent platform to identify potential risk factors, test functional hypotheses, and assess the efficacy of various treatments for NSSI in humans.

Spontaneously occurring SIB has been observed in several species of small mammals (Vergneau-Grosset and Ruel, 2021) and more importantly for the present review, it also occurs in a small percentage of non-human primates living in diverse settings (e.g., research facilities, zoological parks, and natural habitats). Because non-human primate SIB is not experimentally induced, considerable efforts are required to identify the risk factors that lead to this condition. In that sense, these efforts parallel those of clinicians and scientists in characterizing risk and vulnerability in individuals with NSSI. Although spontaneously occurring SIB has been observed in several species of Old World monkeys and great apes, we focus here on a model of this behavioral pathology in rhesus monkeys that has been studied extensively. Below we first identify concordances of rhesus monkey SIB with human NSSI across a number of domains that include form, prevalence, associated features, etiology, and function. We then discuss the value of this animal model for testing potential treatments.

The timing of this evaluation is significant inasmuch as NSSI is under consideration as a distinct disorder (NSSID) in the Diagnostic and Statistical Manual of Mental Health Disorders (DSM-5). However, this proposed classification is not without controversy. Some have argued that NSSI is merely a symptom of other mental conditions, particularly borderline personality disorder and therefore, NSSID has limited value as a distinct diagnostic category (for example, see Ghinea et al., 2020). Others note that NSSI is associated with many different personality disorders and more importantly, it can also occur in non-clinical populations. These arguments support the development of NSSID as a new diagnostic category (for example, see Hooley et al., 2020). This controversy highlights the heterogeneous nature of NSSI, a condition with many potential causes, risk factors, associations, and comorbidities. It would be unreasonable to expect that SIB in monkeys would serve as a model for NSSI broadly. Accordingly, in the present article, we primarily limited the scope of our comparison to the occurrence of NSSI in stress-impacted disorders (especially post-traumatic stress disorder [PTSD]) and in community samples composed of teenagers and young adults with no formal mental health diagnosis. Furthermore, we included information on NSSI in these restricted populations, only as it related to the features present in monkeys with SIB. For some of these features (e.g., hypothalamic pituitary adrenocortical [HPA] axis activity, sleep disruption, and aggressive behavior), the extant literature was small and comprehensive coverage was possible. For other features (e.g., early life stress), the extant literature was so large that comprehensive coverage was not possible. Instead, we relied on recent meta-analyses or systematic reviews. For other aspects such as prevalence rates, the extant literature was so variable, we only attempted to cover the range. Relevant search terms included with NSSI and deliberate self-harm were early life stress, adolescent stress, peer relationships, HPA axis, sleep disruption, insomnia, genetic polymorphisms, genome-wide association studies (GWAS), serotonin, serotonergic, GABAergic, adrenergic, opioids, prevalence, function, and etiology. All relevant papers published prior to February 2021 were examined.

CHALLENGES TO THE STUDY OF SELF-INJURY IN HUMANS AND MONKEYS

Understanding why organisms engage in self-injury is a complicated endeavor, especially when the early patterns and trajectories of this behavior can be discerned or hypothesized only through retrospective analysis of past history and, in the case of humans, using verbal accounts. Two additional issues come into play in any discussion of the etiology of SIB and NSSI. These include both the presence of wide individual differences among monkeys who develop SIB and humans who show a pattern of NSSI, and equifinality, the idea that a given end state, such as self-injury, can be reached through many different pathways.

Individual Differences

Rhesus monkeys housed in research facilities typically differ in their genetic background (Driscoll et al., 2016), temperament (Altschul et al., 2016; Bliss-Moreau and Moadab, 2016), clinical health history (Urvater et al., 2000; Wu et al., 2020), and previous experiences (Dettmer et al., 2012; Morin et al., 2019). As we describe below, some of these individual differences conferred vulnerability to SIB and influenced treatment success. Thus, SIB could not be resolved effectively in all monkeys with a standard treatment regimen. In a similar vein, both the presence and severity of NSSI covary with many different factors such as temperament (Buelens et al., 2020) and early life stress exposure (Cassels et al., 2018).

Equifinality

It is clear from our research that not all monkeys are exposed to the same set of experiences prior to the emergence of SIB. Even if some animals have similar exposures, these experiences may not occur at the same time points. Furthermore, individuals develop at different rates and may not be at the same maturational stage for these variables to induce an effect. This variation in exposure and maturation helps explain not only why some monkeys develop SIB but others do not; it also underscores the principle of equifinality because multiple pathways can lead to the same outcome of SIB. Three diverse examples illustrate this point. First, in the monkeys that exemplify our model,

SIB appeared to result from an interaction between genetic factors, early life stress exposure, and a dysregulation of the stress response system (Novak, 2003; Tiefenbacher S. et al., 2005). Second, SIB developed in some monkeys as a consequence of a clinical disease, reactive arthritis (Urvater et al., 2000). Lastly, a case report identified inadvertent reinforcement by animal care staff for increased attention as the source of SIB in an individual baboon (Dorey et al., 2009).

This concept of equifinality also applies to NSSI, which is associated to a greater or lesser degree with many diverse psychiatric disorders and also occurs in non-clinical populations. Although previously identified as a defining characteristic of borderline personality disorder (Stead et al., 2019), NSSI is also linked to other mental health conditions that include, but are not limited to, depressive disorders (Kaess et al., 2012), autism spectrum disorder (Flowers et al., 2020), substance abuse disorders (Escelsior et al., 2021), eating disorders (Pérez et al., 2018), developmental disabilities (Hoch et al., 2016), and posttraumatic stress disorder (Kimbrel et al., 2016). Not all individuals with these disorders also engage in NSSI. However, comparisons of those that exhibit NSSI vs. those that don't can aid in the identification of the other factors associated with the development of self-injury. For example, individuals with PTSD and a recent history of NSSI were much more likely to make violent threats and act aggressively toward others than PTSD patients without NSSI (Calhoun et al., 2017). In a similar vein, a comparison of two depressed groups – those with NSSI vs. those without – revealed a dysregulation of the HPA axis and elevated perceptions of stress in the NSSI group (Klimes-Dougan et al., 2019). It is unlikely that any animal model, including the one discussed here, could be applicable to all the disorders describe above. Thus, the focus of the present rhesus monkey model as noted above will be on its applicability to the occurrence of NSSI in stress-related disorders, such as PTSD and in non-clinical populations (i.e., community samples of adolescents, college students, military veterans, and other groups that neither have a developmental disability nor have received a formal diagnosis of and/or are under treatment for an anxiety, mood, or psychotic disorder).

CHARACTERISTICS OF SELF-INJURY IN HUMAN AND MONKEYS

Definition and Forms

Human Self-Injury

Non-suicidal self-injury is commonly defined as deliberate body-directed mutilation without suicidal intent that is distinct from socially approved forms of harm such as tattoos or piercings (Nock, 2009). However, studies have also shown a strong connection between NSSI and suicidal thoughts and behaviors, indicating that the presence of NSSI may be a precursor to suicide attempts in some individuals (Whitlock et al., 2013). NSSI can take many different forms including cutting, burning, head banging, biting, hitting/punching, and excoriation of wounds (Klonsky, 2011; Kimbrel et al., 2017; Mann et al., 2020). It is not uncommon for individuals to use more than one method, and the more serious methods may require emergency department visits (Ammmerman et al., 2019).

Monkey Self-Injury

Self-injurious behavior in non-human primates is defined as any self-directed behavior to the body that causes tissue damage in the form of abrasions, cuts, gashes, and punctures of the skin. We use SIB as the descriptor because suicidal intent cannot be inferred from monkey behavior. The most common form of SIB is biting directed to arms and legs and occasionally to the torso. Because of marked sexual dimorphism in the size of the canine teeth in Old World monkeys, males can produce more serious wounds, such as punctures and gashes, than females. Serious wounds require veterinary treatment. Other forms of SIB include hitting, head banging, and disruption of wound healing (Novak, 2003; Taniguchi and Matsumoto-Oda, 2018). It is uncommon for individuals to use multiple methods, except in the case of excessive manipulation of bite wounds that significantly delays healing. However, monkeys may show a preference for particular body sites, leading to permanent skin damage. Although SIB has been studied most extensively in primate research facilities, it also occurs in primates housed in zoological gardens (Hosey and Skyner, 2007) and those living in natural environments (Grewal, 1981; Taniguchi and Matsumoto-Oda, 2018).

Prevalence Rates

Human Self-Injury

The reported prevalence of NSSI, even within specified non-clinical populations, is highly variable and is likely influenced by the methodology used to collect the data (Muehlenkamp et al., 2012). Researchers generally focus on both lifetime prevalence and the current 12-month prevalence. For example, a review and meta-analysis of NSSI in non-clinical populations estimated a lifetime prevalence of 20% in college students (Swannell et al., 2014). A cross-sectional study of first-year students conducted in 2008, 2011, and 2015 found a dramatic increase in lifetime NSSI prevalence from 20 to 45% across the three cohorts (Wester et al., 2018), suggesting that NSSI has become a growing public health concern. However, prevalence rates of NSSI are typically based on self-report and do not take severity or the need for treatment into account. In a sample of 4,565 first-year university students, lifetime prevalence rates were high (nearly one in five individuals), consistent with other findings. The current year prevalence was 9.5%. However, when *DSM-5* criteria, which take into account the severity of the condition, were applied, current year prevalence rates dropped to 0.8% (Kiekens et al., 2018). This difference in reported prevalence rates suggests that the degree of psychological distress and/or impairment associated with NSSI can vary substantially across university students, with only a small proportion of that population showing sufficiently severe symptoms to meet *DSM-5* criteria.

Monkey Self-Injury

Prevalence of SIB in rhesus monkeys and other closely related macaque species can be established both by examining colony health records to identify monkeys who received veterinary

care for self-inflicted wounds and by conducting behavioral surveys of the monkeys in the research colony. Early estimates of the prevalence of SIB derived from a review of colony health records at three major primate facilities revealed a prevalence rate ranging from 11 to 15% of the total research population (Bayne et al., 1995; Bellanca and Crockett, 2002; Lutz et al., 2003b). Assessments were based on the time period the animals were housed in the facility, which is the closest approximation to a lifetime prevalence rate that can be obtained under these conditions. At the specific facility where our intensive SIB research was being conducted, we determined a "lifetime" prevalence rate of 11% (Lutz et al., 2003b). In terms of incidence, monkeys at this facility averaged about 2 major wounding events in a 5-year period (Novak, 2003). Although at first glance this seems like a very low incidence rate, regular behavioral observations of the animals provided a different picture of this disorder. The monkeys actually engaged in self-biting behavior on a daily basis, but serious wounding events requiring veterinary care were infrequent. Instead, these daily biting episodes produced, at most, small cuts or minor skin abrasions (Novak, 2003). Over the years, significant changes in colony management have reduced the "lifetime" prevalence of SIB in primate facilities to a range of 1–5% (Lee et al., 2015).

Distribution Across Age and Sex

Human Self-Injury

Non-suicidal self-injury typically arises in adolescence, with early onset yielding greater vulnerability. Individuals younger than 12 years of age at the time of onset tend to develop more severe forms of NSSI and require more hospital visits (Ammerman et al., 2018). Whether a gender bias exists in NSSI is unclear. In some reports, women appear more vulnerable to this disorder than men (e.g., Favazza and Conterio, 1989), but in others, no gender difference was detected (e.g., Klonsky et al., 2003). A more recent meta-analysis supports the view that NSSI is more common in women than in men, although the difference is greater in clinical populations compared to college/community samples (Bresin and Schoenleber, 2015).

Monkey Self-Injury

Similar to NSSI, SIB in rhesus monkeys appears to arise in adolescence (Lutz et al., 2007). However, unlike the gender difference reported for NSSI, initial findings suggested that SIB was much more common in males than in females (Lutz et al., 2003b). More recent surveys of colony health records failed to detect any sex difference (Peterson et al., 2017), but there may be a male bias in severity, perhaps explained by the presence of enlarged canine teeth in males.

Similarities and Differences in Human NSSI and Rhesus Monkey SIB

The information presented above suggests substantial concordances between human NSSI (as seen in non-clinical populations) and the model of rhesus monkey SIB. The first key similarity is that the form of self-harm (e.g., biting, hitting, head banging, skin gouging, etc.) is highly congruent across humans and monkeys, excluding cutting and burning for which there is no monkey equivalent. Second, both disorders arise spontaneously, without the need for induction. Third, both disorders typically emerge in adolescence, suggesting that certain developmental events play a role in the emergence of both NSSI and SIB. Finally, there is a continuum of severity ranging from milder cases to the more severe forms of wounding, requiring extensive veterinary care in monkeys and hospitalization in humans. Some disparities also exist. Gender differences are not the same. Whereas women are at somewhat higher risk in developing NSSI than men, recent research on monkeys suggests either a slight male bias or no difference between males and females. Additionally, prevalence rates of self-injury are generally lower in monkeys than in humans except when *DSM-5* criteria are applied. Note, however, that the gradual identification of risk factors for SIB (described below) has been used by primate facilities to reduce its prevalence, whereas prevention strategies have yet to be developed for people who are at risk for NSSI (Brown and Plener, 2017). In the remainder of this paper, we will provide more details about our specific model of rhesus monkey SIB, and we will discuss how information about the etiology of the disorder, its biological underpinnings, and potential treatments may inform the study and treatment of NSSI in humans.

THE MODEL OF SELF-INJURY IN RHESUS MONKEYS

Approach

This model focuses on the spontaneous occurrence of SIB in rhesus monkeys in contrast to animal models in which the abnormal behavior is induced experimentally by genetic manipulations or drug treatments (e.g., amphetamine). The consequence of studying spontaneously occurring disorders is that strict causality cannot be evaluated. Instead, this model is based on the identification of risk factors and correlates. Risk factors are those features that increase the probability of a monkey's chance of developing SIB. By definition, such factors must have predictive value and be present before the disorder appeared, as determined by historical data (Offord and Kraemer, 2000). Some risk factors are fixed (e.g., genotype, rearing practice) whereas others are variable (e.g., length of individual cage housing). In contrast, correlated features are concomitant with the disorder, determined through observations of monkeys with SIB in comparison to healthy controls. In the latter case, the sequential relationship between these features and SIB cannot be determined.

We employed two strategies to understand the etiology of SIB in rhesus monkeys. First, risk factors were identified by examining the colony health and management records and comparing monkeys having a veterinary record of SIB (i.e., at least one self-inflicted wounding episode that required veterinary attention) to monkeys that had no such record. The data set consisted of 362 animals with similar housing. Relevant information in the records included early rearing practice, housing history, health assessments, number of relocations, and number of veterinary procedures (e.g., blood draws). Variables

were created from the information and statistically analyzed to identify risk factors for SIB. A similar procedure was carried out at other primate facilities, and commonalities across facilities were noted. Additionally, a subset of monkeys with and without SIB was genotyped for specific genetic polymorphisms previously related to various mental health conditions.

In the second strategy, the same subset of monkeys both with and without SIB were observed and examined intensively to determine the presence of behavioral or physiological features associated with SIB. This approach enabled us to show that the disorder in rhesus monkeys is a syndrome consisting of multiple abnormalities, not just self-injury.

Features of the Model

We hypothesize that SIB emerges in some monkeys from an interaction between adverse early experience and a genetic predisposition, leading to a dysregulation of the neuroendocrine stress response (Tiefenbacher et al., 2004). Although the mechanism underlying such dysregulation is not yet clear, one intriguing possibility involves epigenetic modifications of glucocorticoid receptor gene expression (Matthews and McGowan, 2019). These factors interact to sensitize the animals to additional stress exposure and subsequently lead to disturbances in other neurobiological systems that manifest as increased aggressiveness (Lutz et al., 2003a), and sleep disruption (Stanwicks et al., 2017). The development of SIB is then viewed as a coping response to ongoing stress exposure.

Risk Factors: Early Life Stress, Neuroendocrine Stress Dysregulation, and Genetic Polymorphisms

Colony Record Data and Links to Early Life Stress

A statistical analysis of the colony health and management records of large populations of monkeys at different primate centers revealed the importance of various life stressors in determining the risk for developing SIB. The most significant risk factor for this disorder was maternal separation of infants at birth followed by nursery rearing. In one study focusing on this specific factor, the ratio of nursery-reared monkeys to maternally reared monkeys that developed SIB was 14:1 (Lutz et al., 2007). The adverse consequences of nursery rearing have also been reported at four other primate centers (Bellanca and Crockett, 2002; Lutz et al., 2003b, 2007; Rommeck et al., 2009; Gottlieb et al., 2013). However, even mother-reared monkeys developed SIB if they experienced stressful events as juveniles. One such stressful event was going onto a research protocol that entailed separation from the social group during this crucial developmental period. Thus, monkeys that developed SIB were separated as juveniles (average age of 14 months) whereas monkeys that remained healthy were separated as adolescents (average age of 36 months) (Lutz et al., 2003b). Finally, some monkeys developed SIB in adulthood which was linked to cumulative stress exposure. The odds of developing SIB in adult monkeys increased with the number of veterinary procedures requiring restraint and sedation (e.g., blood draws) and with the length of time monkeys were individually housed for research purposes (Lutz et al., 2003b; Gottlieb et al., 2013).

Neuroendocrine Stress Mechanisms

Features of the HPA axis

The hypothalamic-pituitary-adrenocortical (HPA) axis plays a significant role in both the response and recovery to acute stressors. However, when stressors are chronic or cumulative, HPA axis function may become dysregulated. Most studies measure cortisol, the principal output of the HPA axis in humans and non-human primates. Cortisol levels can be measured in many different bodily fluids and tissues, including blood, urine, saliva, feces, sweat, hair, and fingernails. As noted in **Table 1**, these sample matrices differ with respect to the time frame they represent, the cortisol fraction being measured, units of measure, and susceptibility to being influenced by circadian rhythms (Novak et al., 2013; Meyer and Novak, 2021).

The selection of sampling matrix depends on the objective of the study. Thus, blood and saliva samples are most appropriate for assessing the time course of HPA responses to acute stressors, whereas hair and fingernails are more relevant for measuring the impact of long term stressors. It should also be noted that the relative concentrations within each matrix are very different. For example, plasma samples contain total cortisol and are many times higher than salivary cortisol samples, which reflect only the free (unbound) cortisol fraction.

Role of the HPA axis in SIB

We hypothesized that the stress of early maternal separation was a key factor in the increased vulnerability to SIB in nursery-reared compared to mother-reared monkeys. If this hypothesis was correct, then nursery-reared infant monkeys might show altered

TABLE 1 | Characteristics of various cortisol sampling matrices.

Matrix	Time Frame	Cortisol Fraction	Unit of Measure*	Subject to Circadian Variability
Blood (plasma or serum)	Minutes	Total (free and bound)	Mass per unit volume	Yes
Saliva	Minutes	Free	Mass per unit volume	Yes
Urine	Hours/Day	Free + (creatinine correction for urine volume)	Mass per unit volume	Yes (unless 24 h urine collection)
Feces	Hours/Day	Free	Mass per unit weight	Yes (unless 24 h fecal collection)
Hair	Months	Free	Mass per unit weight	No
Fingernails	Months	Free	Mass per unit weight	No

**Refers to how sample cortisol concentration is expressed (e.g., ng/ml for plasma cortisol versus pg/mg for hair cortisol).*

HPA axis activity compared to a mother-reared infant group. In two separate studies, nursery-reared monkeys showed lower plasma cortisol concentrations in response to the acute stress, involving a brief separation from their social group for blood sampling and temperament testing, compared to maternally reared monkeys who underwent the same procedures (Capitanio et al., 2005; Kinnally et al., 2008). The blunted stress response in nursery-reared infant monkeys was also present in juveniles with early life stress exposure (Feng et al., 2011). Because this blunted cortisol response initially occurred in infants prior to the typical age of SIB onset, we consider dysregulated HPA axis activity to be a risk factor for this condition.

Studies of adult monkeys have lent additional support for this view. Compared to controls, monkeys with SIB also showed a blunted plasma cortisol response to the acute stress of capture and blood sample collection (Tiefenbacher et al., 2000, 2004). Additionally, plasma cortisol concentrations were negatively correlated with biting rate and with recency of a serious wounding event. In contrast to this blunting effect, chronically *elevated* HPA activity, assessed by cortisol accumulation in hair samples, was observed in SIB monkeys compared to healthy controls (Davenport et al., 2008). These findings are best explained by the presence of persistent long-term HPA activation leading to a compensatory dampening of the normal cortisol rise in response to an acute stressor (Herman et al., 2016). In this form of HPA axis dysregulation, the organism is unable to mount an adequate response to short-term stressful events.

Role of Genetics in Stress Dysregulation and SIB

For many years, genetic contributions to complex human behaviors in general and psychiatric disorders in particular were examined using a candidate gene approach in which genes considered likely to be associated with the behavior or disorder were selected *a priori* for study. Because of the limitations of this approach, which include the obvious selection bias, the candidate gene approach has largely been replaced with GWAS. To our knowledge, SIB in monkeys has not yet been investigated using the GWAS approach; however, several earlier studies were conducted relating specific genetic polymorphisms to SIB and to HPA axis function. We reasoned that because most monkeys subjected to nursery rearing (an early life stress) did not develop SIB, the animals that did show the behavioral pathology may have possessed one or gene alleles that conferred vulnerability. To test this hypothesis, a smaller subset of monkeys with and without SIB was genotyped for the presence of several genetic polymorphisms previously associated with various psychiatric conditions. We specifically investigated the following three genetic polymorphisms: the serotonin transporter gene-linked polymorphic region (5-HTTLPR), a single nucleotide polymorphism in the mu-opioid receptor gene (C77G), and regulatory polymorphisms of the tryptophan hydroxylase-2 (TPH2) gene. Only the latter two polymorphic variants were associated with the features of SIB. The mu-opioid receptor variant (G77-containing alleles) was associated with higher beta-endorphin affinity, lower blood cortisol levels in response to an acute stressor, and more aggressive threat behavior in rhesus monkeys (Miller et al., 2004). Additionally, the distribution of rhTPH2 5′-FR haplotypes differed significantly between monkeys with and without SIB, and this genetic difference was also associated with lower plasma cortisol concentrations in response to acute stress exposure (Chen et al., 2010). Although these findings are consistent with the general theory that spontaneously occurring SIB in monkeys likely involves gene by environment interactions, the specific associations discussed here must be considered preliminary given the small sample sizes and lack of replication to date. GWAS based on a larger population of monkeys with and without SIB is needed if we are to advance our understanding of the role of genetics in this disorder.

Behavioral Correlates of SIB

As mentioned earlier, we hypothesize that spontaneously occurring SIB in rhesus monkeys is one component of a broader constellation of behavioral and neurobiological abnormalities. To test this hypothesis at the behavioral level, we compared SIB and non-SIB monkeys with respect to differences in anxious behavior, social behaviors, and sleep.

Anxious Behavior

Typical forms of anxious behavior in macaque monkeys include yawning, scratching, vigilance, and displacement activity. Baseline levels of these behaviors in the home cage did not vary across monkeys with and without SIB. However, anxious behavior can also be assessed with various experimental tests, one of which is the Human Intruder Test (HIT). In this test, an unfamiliar human approaches a monkey, stands in profile, turns to gaze at the monkey, then turns her back, and then finally exits the room. Anxious behavior includes moving away or looking away from the intruder and showing fear responses, especially during the gaze portion of the test. Consistent with the view that heightened anxiety was not a key feature of this disorder, monkeys with SIB showed *lower* levels of anxious behavior during the HIT compared to controls (Peterson et al., 2017). Anxious behavior has been associated with reduced serotonergic activity and with the serotonin transporter gene (5-HTTLPR) short allele. Serotonergic activity in monkeys with and without SIB was evaluated by measuring the concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid in cerebrospinal fluid, by determining the prevalence of the 5-HTTLPR short allele, and by evaluating the serotonergic response to a fenfluramine challenge test. No relationship to SIB was detected in any of these measures, indicating that if serotonin plays a role in the disorder as suggested by the TPH2 gene results, functional dysregulation of the serotonergic system has not yet been identified (Tiefenbacher et al., 2000, 2003; Tiefenbacher S. et al., 2005). Because of this negative outcome, we did not evaluate anxious behavior in humans with NSSI.

Deficits in Social Interaction

Although not abnormally anxious, monkeys with SIB showed deficits in social interactions with other monkeys as manifested by high levels of aggressive behavior and low levels of affiliative behavior compared to monkeys without SIB. Biting and aggressive behavior were linked in that 5-min observational samples containing biting episodes had a higher proportion

of threats to other nearby monkeys than non-biting samples (Novak, 2003). The act of biting is a species-typical behavior, since monkeys typically bite and wound other monkeys in aggressive altercations. Thus, our findings raised the general question, were self-directed bites a form of redirection to oneself when physical contact with others was prevented?

Threatening behavior was subsequently manipulated by exposing monkeys with and without SIB to an unfamiliar monkey in a different pen. Although the manipulation increased threatening behavior in monkeys with SIB, it did not alter their biting rates (Lutz et al., 2003a). These findings indicate that SIB in rhesus monkeys is not merely redirected aggressive behavior. Thus, the functional relationship between aggression and biting behavior in monkeys requires further study.

Sleep Disruption

In early studies of nighttime activity, monkeys with SIB spent more time awake at night than controls (Davenport et al., 2008). These findings were later replicated in a different population of monkeys with and without SIB using infrared surveillance software that could reveal the timing of this activity as it occurred across the night. Using this approach, we found that monkeys with SIB compared to controls showed delayed sleep onset, higher overall activity at night, and longer bouts of activity when awake (Stanwicks et al., 2017). Although these activity data are not definitive with respect to sleep since there was no monitoring of cerebral EEG, the results strongly suggest that several aspects of the sleep cycle (e.g., greater latency to sleep onset and less overall sleep) are abnormal in monkeys with SIB.

THE RHESUS MONKEY MODEL: PARALLELS ACROSS RISK FACTORS AND CORRELATES IN HUMANS WITH NSSI

One of the limitations of our model is that, unlike humans, monkeys cannot directly reveal their feelings or thoughts. Thus, some of the very important correlates of NSSI such as negative affect, dissociative states, and other internalizing symptoms are obviously beyond the scope of this model. Our etiological model of rhesus monkey SIB emphasizes the importance of early life stress combined with genetic vulnerability and HPA axis dysregulation to evoke SIB and other associated features, such as deficits in social interaction and sleep disruption.

A number of human etiological models have been developed to explain how various factors may interact to produce NSSI. In one of these models, Nock (2009) has argued that early life stressors (particularly child abuse) interact with genetic risk factors for deficient cognitive processing and abnormal emotional reactivity. This interaction is then hypothesized to lead to emotion dysregulation and inadequacies in communication, thereby increasing vulnerability to NSSI. In the following section, we consider the extent to which the factors thought to underlie NSSI in humans parallel the risk factors and correlates of SIB in our rhesus monkey model.

Risk Factors for NSSI

Early Life Stress

As in the monkey model, early life stress was a significant predictor of NSSI within both community (Yates et al., 2008; Taliaferro et al., 2012) and clinical samples (Kaess et al., 2013). This connection was further supported by a recent systematic review of 20 cross-sectional studies (Serafini et al., 2017). In nearly all of these studies, the development of NSSI was linked to some form of early childhood adversity.

As noted in the monkey model, the impact of stress was not limited to childhood. In females, the risk of developing NSSI increased with each additional stressful event after a baseline of two such events (Steinhoff et al., 2020). NSSI can also develop in adults after exposure to a single highly stressful event. Of the men and women who experienced military sexual assault, over 25% engaged in NSSI; and within that population, the majority indicated that their first act of NSSI occurred after the traumatic event (Holliday et al., 2018).

Neuroendocrine Stress Response

There is now growing evidence that HPA axis activity is dysregulated in individuals with NSSI. In these studies, blood or salivary cortisol has typically been measured in response to an acute stressor; only occasionally has hair cortisol been assessed to evaluate chronic stress exposure. As observed in rhesus monkeys, individuals with NSSI showed a blunted response to an acute stressor. In the most recent study, Reichl et al. (2019) compared adolescents engaging in NSSI with their siblings. Adolescents with NSSI reported more childhood adversity and showed a blunted salivary cortisol response to a trauma interview in which they were asked to relive traumatic events. The authors also assessed chronic stress exposure and found hair cortisol concentrations to be elevated in the NSSI group when the data were controlled for smoking behavior. Two other acute stressors have been tested for their effects on salivary cortisol. Individuals with NSSI showed a blunted salivary cortisol response to the Trier Stress Test (Kaess et al., 2012; Klimes-Dougan et al., 2019) but elevated salivary cortisol in response to the cold pressor task (Koenig et al., 2017). It is noteworthy that this elevated cortisol response occurred to a non-psychosocial stressor, whereas the previously mentioned studies that reported blunted cortisol responses used psychosocial stress challenges. Finally, other features of HPA axis dysregulation in people with NSSI have been shown by elevated salivary cortisol concentrations 30 min after awakening (i.e., cortisol awakening response) (Reichl et al., 2016) and a substantially greater suppression of blood cortisol concentrations in response to a dexamethasone suppression test (Beauchaine et al., 2015).

Role of Genetics in NSSI

The study of genetic involvement in NSSI suffers from the same limitations as discussed earlier for monkey SIB. A number of reports of GWAS in relation to suicidality have been published; however, to date the only application of this approach to NSSI is the recent study by Campos et al. (2020) that looked for genes related to self-harm behavior and/or self-harm ideation. Although several genes were significantly

associated with one or both of these variables, the one with the strongest association with both was *dcc* (deleted in colorectal cancer), a gene known to play an important role in the development of the prefrontal cortex (Manitt et al., 2013). Much more research is needed to understand how genetic variation is related to NSSI versus suicidality, especially since self-harm ideation and behavior leads to suicide attempts in some individuals.

Considering the genetic polymorphism studies that have been associated with monkey SIB or its correlated features, it seems reasonable to mention any prior studies of NSSI involving the same genes to determine if parallel findings were obtained. Our literature search revealed that only one of the genes associated with SIB has also been studied with respect to NSSI, namely the TPH2 gene. In this case, however, the genetic variants have been linked specifically to the development of suicide risk, not to NSSI (see meta-analysis by Ottenhof et al., 2018). Nevertheless, this finding is of interest given the substantial evidence that NSSI is a gateway to suicide (Griep and MacKinnon, 2020). Also related to the serotonergic system is a study of the serotonin transporter gene length polymorphism (5-HTTLPR) in NSSI. The polymorphic variants consist of a short and a long allele, of which the short allele is associated with lower levels of serotonergic activity. Hankin et al. (2015) found that individuals who carried the short allele and experienced chronic interpersonal stress showed high levels of NSSI. Consistent with the genetic results is a report of lower plasma serotonin concentrations in a community sample of self-injuring adolescents compared to controls (Crowell et al., 2008). Although we found no association between 5-HTTLPR and SIB in monkeys, it remains possible that this was because of the small sample size or other mitigating factors.

Correlates of NSSI

In our monkey model, SIB was significantly associated with deficits in social communication, manifested primarily as aggressive and threatening behavior, and sleep disruption. In general, NSSI has been associated with many different features, the presence of which varies considerably across individuals. Below, we assess the two primary correlates identified in monkey SIB and determine their potential role in NSSI.

Aggressive Behavior

An extensive literature supports an association of NSSI with other-directed aggression both in high school students (Brunner et al., 2007) and clinical populations (Boxer, 2010). The association between aggressive behavior and NSSI has been shown to persist in adolescents and university students even when controlling for the effects of emotion regulation (Tang et al., 2013; Sorgi et al., 2020). One form of aggression in teenagers is bullying behavior, and NSSI is associated with frequent victimization (Fisher et al., 2012; Jantzer et al., 2015). However, it has also been linked to perpetrators of bullying (Esposito et al., 2019). Stress-related disorders appear to mediate the relationship between aggression and NSSI in some individuals. Veterans with PTSD who also engaged in NSSI were more likely to threaten and commit violence against others than veterans with PTSD but without NSSI (Calhoun et al., 2017).

Is NSSI in aggressive individuals a form of aggression toward self? This intriguing idea is supported by the finding that aggressive individuals tend to engage in specific forms of NSSI, namely hitting and punching oneself. Kleiman et al. (2015) found that trait aggression was associated both with the form of NSSI expression and with a lifetime history of self-hitting behavior. Hitting does not have to be directed to the self to be injurious. Wall/object punching was common among war veterans diagnosed with PTSD (43%). This behavior resulted in injuries and yielded the typical post-injury relief associated with the commission of other forms of NSSI (Kimbrel et al., 2017).

Sleep Disruption

Self-injurious behavior in rhesus monkeys was associated with significant sleep disturbances including delayed sleep onset, shorter sleep length, and more awakenings per night. Parallel to the monkey model, recent studies have suggested a strong relationship between NSSI and sleep problems. Nearly all of these studies rely on self reports of sleep quality using various scales. They do not measure sleep directly with polysomnography or examine nighttime activity patterns using actigraphy or surveillance software. Regardless of this limitation, virtually all of these studies revealed a pattern of disrupted sleep. The most common forms of dysregulated sleep endorsed by individuals with NSSI were insomnia, nightmares, and poor sleep quality. Insomnia was significantly associated with NSSI in a community sample of adolescents, an effect that persisted after controlling for depressive symptoms (Latina et al., 2021), and also in a survey of Norwegian adolescents (Hysing et al., 2015). Insomnia symptoms were additionally linked to the recency of NSSI (Bandel and Brausch, 2020). In some cases, insomnia was influenced by mental health conditions and/or demographic factors and was not independently associated with NSSI (see Ennis et al., 2017 for a university sample; see Liu et al., 2017 for an adolescent sample). However, in these same two studies, NSSI was independently associated with a different form of sleep disruption, namely nightmares. Nightmares were also a major form of sleep disruption endorsed by the parents of young children with NSSI (Singareddy et al., 2013). Poor sleep quality, without specific reference to the forms of sleep disturbance, has additionally been linked with NSSI (Liu et al., 2017; Asarnow et al., 2020). In the one study to date in which sleep was monitored by polysomnography, only one variable differentiated children with and without NSSI. The percentage of time spent in REM sleep was greater in the NSSI group (Singareddy et al., 2013).

The findings described above demonstrate that aggression and sleep disruption, two behavioral features present in monkeys with SIB, are also frequently found in people with NSSI. These parallels suggest that the common risk factors for SIB in monkeys and NSSI in humans may work through closely related neurobiological pathways to yield similar multi-faceted behavioral syndromes.

FUNCTION: WHAT PURPOSE DOES SELF-INJURY SERVE?

Rhesus Monkey SIB and the Environment

One of the more perplexing challenges has been to understand the why of monkey SIB. Why do monkeys harm themselves and why does this deviant behavior continue to persist in individual monkeys despite intervention attempts? From a broad functional perspective, the commonly held view is that animals engage in abnormal behavior because of powerful reinforcement contingencies. A number of functional models have been proposed to explain what those reinforcement contingencies might be.

In monkeys, we examine how SIB might be used to mitigate the effects of adverse environments. Two hypotheses suggest that SIB serves to ameliorate the ongoing and long lasting effects of adverse environments. Both focus on the extreme ends of a continuum of environmental quality ranging from environments that are under-stimulating and impoverished to those that are over-stimulating and chaotic. Superimposed on this continuum is the presence of wide individual differences in reactions to environments, which poses a barrier to effective treatment.

Impoverished Environments and Sensation Seeking

According to this hypothesis, SIB provides a form of sensory input to an animal existing in a barren environment with little stimulation. As a corollary, SIB is a manifestation of a species-typical response, namely threatening/aggressive behavior, which is thwarted in the absence of social partners and instead redirected to the self. Thus, SIB is positively reinforcing by providing increased stimulation and allowing for the expression of aggressive behavior. If this hypothesis is true, then changing the environment by addressing sensory-perceptual needs and providing adequate social partners should lead to a reduction in SIB. Currently, there is some support for this hypothesis. Providing social partners resulted in a reduction in SIB in some monkeys that previously had been housed in individual cages because of the requirements of specific research projects (Weed et al., 2003; Fontenot et al., 2006). In contrast, however, enhancing the inanimate features of the sensory-perceptual environment was not a particularly effective strategy. Biting behavior rates were unaffected by the addition of manipulable objects and foraging devices (Rommeck et al., 2009), puzzle feeders (Novak et al., 1998), or play cages (Griffis et al., 2013). To put these latter findings in perspective, it is likely that strong individual differences in preferences for various environmental enhancements may have obscured the effectiveness of these changes on reducing SIB.

Stressful Environments and Arousal Reduction

This hypothesis posits that monkeys bite themselves to reduce their arousal in response to stressful stimuli or, as a corollary, to focus their actions inward as a way of avoiding the stressors in their environment. In this scenario, SIB is maintained through a process of negative reinforcement. Some limited support for this view comes from the finding that heart rate patterns in 10 monkeys with SIB were elevated just prior to a biting episode and returned to baseline shortly thereafter (Novak, 2003). A more general test of this hypothesis requires identifying the stressors to which individual animals might react, keeping in mind wide individual variation, and then developing a strategy to mitigate that stress exposure and observing corresponding reductions in SIB.

One of the more effective ways to reduce the stressfulness of various research and veterinary procedures is through familiarization and positive reinforcement training. Monkeys have been trained to give blood samples by freely extending their legs (Graham et al., 2012) and provide saliva samples by chewing on dental rope (Lutz et al., 2000). Additionally, positive reinforcement training has been used to acclimate monkeys to transport boxes and restraint devices (Mason et al., 2019). At the present time, there are no studies assessing the effects of such training on SIB; but there is strong evidence that training is associated with a reduction in other forms of abnormal behavior in monkeys such as repetitive stereotyped behaviors (Baker et al., 2009; Coleman and Maier, 2010).

There are two limitations to the idea that SIB can reduce the effects of adverse environments. First, environmental quality is a construct and does not take into account the dynamic temporal fluctuation in environmental stimulation that occurs in any environment. Additionally, it is likely that motivations for SIB vary both across individuals and within an individual over time.

Human NSSI

Many different theoretical models have been proposed to explain why humans injure themselves. There is evidence for the involvement of the opioid system and for the role of the environment broadly defined. And as noted with the rhesus monkeys, some of these models posit that NSSI is a form of positive reinforcement and others link NSSI to negative reinforcement. More importantly, new advances now allow scientists to obtain information that bears directly on the act of self-injury. Using ambulatory assessment, self-report information as well as behavioral and physiological measures can be obtained from the individuals in real time immediately prior to and directly after episodes of NSSI (Trull and Ebner-Priemer, 2013).

Environmental Factors

A variety of factors elicited through an individual's interaction with their environment can provoke episodes of NSSI. As described in self reports, these episodes are reinforcing, at least temporarily, leading to an ongoing cycle of NSSI-elicited relief, followed by reinstatement of the original problem, leading to more bouts of NSSI. The four function model proposed by Nock and Prinstein (2004) organizes these factors along two dimensions: intrapersonal (e.g., emotion regulation) vs. interpersonal (e.g., social interaction), and negative vs. positive reinforcement. At an intrapersonal level, NSSI may produce negative reinforcement by reducing a negative internal state (e.g., anxiety) or produce positive reinforcement by increasing stimulation in a monotonous environment (Nederkoorn et al., 2016). At the interpersonal level, NSSI may be negatively

reinforcing by reducing unwanted interactions with others, or conversely it may be positively reinforcing by increasing attention to the individual and enhancing social support.

The four function model has been used successfully to classify the forms of NSSI committed by incarcerated populations (Power et al., 2016) and by Swedish community members (Zetterqvist et al., 2013). However, in one study, intrapersonal functions of NSSI were emphasized more and were more likely to produce relief than interpersonal functions (Brausch and Muehlenkamp, 2018). Moreover, a systematic review of 35 ambulatory assessment and diary studies provided support for only the negative intrapersonal function (Taylor et al., 2018; Hepp et al., 2020). These differing results may not be surprising given that the relative importance of these four functions most likely differs between individuals and within individuals over time. It does suggest that a major reason for engaging in NSSI is to reduce negative states of various kinds as has been proposed in the Experiential Avoidance Model (Chapman et al., 2006) and the Emotional Cascade Model (Selby et al., 2013).

The parallels between the monkey and human data are less clear with respect to how an interaction with the environment elicits self-injury. The strongest potential alignment between the monkey and human data comes from the view that in humans, NSSI can reduce adverse mental states triggered by external situations, and in monkeys, SIB may reduce heightened arousal caused by stressful environmental events. One significant advantage in the monkey studies is the ability to redesign environments to mitigate stress exposure and to assess the possible impacts on SIB.

RESOLVING SELF-INJURY

Considerable effort has been expended in developing effective treatments for self-injury in both human and non-human primates. In monkeys, this effort involves environmental redesign and stress reduction procedures (as mentioned in the section under function) as well as pharmacotherapy. In fact, one of the significant advantages of the monkey model is in evaluating the efficacy of new treatments, whether it be environmental, pharmacological, or some combination, in overcoming SIB, prior to their implementation in humans.

Pharmacological Treatments for SIB

Pharmacotherapy is considered a treatment of last resort for non-human primates and is primarily used to treat serious instances of SIB that result in repetitive wounding episodes. Although pharmacotherapy has efficacy in reducing SIB, there are potential risks to health and well-being. These risks include side effects, such as sedation and short-term gastrointestinal distress, development of tolerance requiring escalation of dosing schedules, withdrawal symptoms on dose reduction, and relapse once the drug has been removed. The ideal drug should have three properties: (1) the side effects should be short lasting, (2) the drug should induce changes in brain neurochemistry leading to a major reduction if not a cessation of SIB, and (3) the neurochemical changes and therapeutic benefits should persist after the treatment ends. Over the past 20 years, drugs that alter the activity of the GABAergic, serotonergic, adrenergic, or opioidergic neurotransmitter systems have been tested for their efficacy in reducing SIB in monkeys. To date, none of the tested compounds fits the ideal therapeutic profile, but some drugs come closer than others. In the following section we review each of the systems mentioned and associated drug therapies in order from least effective to most effective.

GABAergic System

Historically, the $GABA_A$ receptor-positive allosteric modulator, diazepam, was used to treat SIB in monkeys, based on its anxiolytic properties. However, subsequent evaluations revealed marked individual differences in response to this drug. For example, when administered to eight monkeys with SIB, only four showed a reduction in wounding rates; the remaining monkeys actually got worse (Tiefenbacher S. T. et al., 2005). Because of this disparate reaction, diazepam is no longer recommended for alleviating SIB in rhesus monkeys.

Serotonergic System

Selective serotonin reuptake inhibitors (SSRIs) have also been used to manage episodes of SIB in rhesus monkeys. Administration of the SSRI fluoxetine and the 5-HT1A receptor partial agonist, buspirone, led to a 50% reduction of SIB during the early weeks of treatment (Fontenot et al., 2005). The post-treatment period was too short (2 weeks) to assess relapse. In a second study, fluoxetine was more effective than venlafaxine (a combined serotonin and norepinephrine reuptake inhibitor; SNRI) in managing SIB. This study only evaluated dosing strategies and did not follow the monkeys post treatment (Fontenot et al., 2009). Serotonergic activity can also be manipulated by adding the precursor L-tryptophan to the diet. In rhesus monkeys, SIB decreased during L-tryptophan administration, but the monkeys relapsed once the dietary supplementation ended (Weld et al., 1998).

Adrenergic System

Guanfacine, an α2A adrenergic receptor agonist, was used to treat SIB in two macaques and one baboon. Self-biting behavior was eliminated by administration of guanfacine; however, the three animals eventually relapsed after the treatment ended (Macy et al., 2000). A wound scoring scale was used in a second study to evaluate the effect of guanfacine on self-inflicted wounds in rhesus monkeys (Freeman et al., 2015). Treatment reduced the severity of inflicted wounds and this effect persisted into a post-treatment period for about 100 days before relapse occurred in some monkeys.

Opioidergic System

To date, the most promising treatment for SIB is the long-acting opioid receptor antagonist naltrexone because it affects not only SIB but also some correlated brain anomalies. Administration of naltrexone reduced SIB during treatment and had long lasting benefits that were still present 110–200 days post treatment (Kempf et al., 2012). In a follow-up study, the postmortem brains of SIB monkeys treated with naltrexone, untreated SIB monkeys, and healthy controls were examined. The results

revealed significant immune activation and atrophy of both gray and white matter astrocytes in untreated SIB monkeys compared to healthy controls. Moreover, these differences were abolished in monkeys that received naltrexone treatment (Lee et al., 2015). Although the sample sizes were relatively small in the two studies, taken together they are consistent with the hypothesis of opioidergic system involvement in SIB in rhesus monkeys. While on board, naltrexone presumably blocked the reinforcing effects of self-biting-induced opioid release, leading to an extinction of the behavior. The mechanism by which this extinction continued even after the end of treatment is unknown; however, the neuropathological findings suggest that a reversal of CNS immune activation and astrocyte atrophy may have played some role in this effect.

Pharmacological and Psychotherapeutic Treatments for NSSI

Pharmacological Approaches

Typically, psychotherapeutic approaches are more commonly implemented in the treatment of NSSI than pharmacological approaches. In part, this difference may be related to the potential risks to health and well-being associated with the adverse reactions of some individuals to drug treatments developed for mood and stress-related disorders. As in rhesus monkeys, these reactions can include side effects such as sedation, development of tolerance, and withdrawal symptoms upon removal of the treatment. Certain drugs can also increase the risk of suicidal ideation (Zisook et al., 2009), and some individuals may either drop out or show poor compliance in taking their medication. Lastly, individual differences in drug response are difficult to predict and manage, and relapse (a reappearance of NSSI) may occur after the treatment is discontinued.

Despite these concerns, drug treatment may be necessary to treat very severe forms of NSSI. Some empirical evidence exists to support the efficacy of pharmacotherapy in reducing or eliminating NSSI. In common with the monkey studies are the use of SSRIs (fluoxetine), SNRIs (venlafaxine), and opioid antagonists (naltrexone). In addition, two atypical antipsychotics (aripiprazole and ziprasidone) have yielded some efficacy in reducing NSSI. In most cases, pharmacotherapy involved patients with borderline personality disorder. Borderline patients showed a reduction in NSSI with fluoxetine (Markovitz et al., 1991), venlafaxine (Markovitz and Wagner, 1995), and naltrexone (Sonne et al., 1996). In the latter study, patients were treated with naltrexone for only 1 week followed by a 1-week post-treatment period in which all five patients experienced relapse. However, the short period of treatment was probably not sufficient to establish drug efficacy. In another patient population, seven females diagnosed with borderline personality disorder received naltrexone for their clinically significant SIB. Naltrexone eliminated NSSI in six of seven patients during treatment. Two patients, who had discontinued naltrexone, showed a resumption of NSSI, which was eliminated when the naltrexone was reinstated (Roth et al., 1996). In the only randomized clinical trial with borderline patients, the atypical antipsychotic, aripiprazole, led to a cessation of NSSI which persisted for 18 months after treatment in most of the drug treated group compared to the placebo group (Nickel et al., 2006, 2007).

These studies suggest that several different kinds of medications may have acute beneficial effects on NSSI. It is possible that at least in some cases, reductions in self-harm are related more to the ability of the medication to temporarily alleviate the patient's dysphoric state, rather than targeting the specific mechanisms underlying the behavioral pathology. Most importantly, much more research needs to be done to determine the ability of pharmacotherapeutic interventions to produce lasting reductions in NSSI post-treatment.

Psychotherapeutic Approaches

Many different psychotherapeutic approaches have been tried as a treatment for NSSI. A review of all possible psycho-social interventions is outside the scope of this review, particularly because such interventions are difficult to model using non-human primates. We restrict our brief discussion to a recent meta-analysis and a systematic review of randomized control trials of various treatment interventions. A meta-analysis of 25 randomized control trials revealed that most treatments yielded only small or moderate effect sizes when compared to active controls. The most noteworthy approach was dialectical behavior therapy targeted for adolescents (DBT-A) which had moderate effects in reducing NSSI (Kothgassner et al., 2020). In a systematic review, Glenn et al. (2019) noted that currently, there are few Level 1 "well established" interventions for NSSI, with one exception being DBT-A. In both studies, the authors point to the need for independent replication of treatments that currently fall into the category of Level 2 "probably efficacious" in reducing NSSI.

Some of the problems associated with pharmacotherapy as a treatment also pertain to psychotherapy. Individual reactions to psychotherapy can be difficult to predict and may require alteration in therapy protocols. Some individuals may show low compliance with protocols. Furthermore, reinstatement of NSSI may occur once the therapy is discontinued. All of this points to the need for new interventions and prevention strategies, particularly in the case of severe, unremitting NSSI.

DISCUSSION

In contrast to various genetic, physiological, and infectious diseases, mental health disorders are challenging to model in animals. Many of the symptoms are subjective in nature and are identified primarily through self-report. Additionally, relevant biomarkers that could be modeled in animals are often lacking. Nor are simple diagnostic tests available that can determine whether an individual has or does not have a mental health disorder (Nestler and Hyman, 2010).

Non-suicidal self-injury is somewhat different than other mental health disorders in that its starting point is a group of behavioral acts that yield a discernible physical outcome of tissue damage. That aspect can clearly be modeled in non-human primates, some of whom develop a pathology in which they bite themselves to the point where they require veterinary care.

However, the many varied functional explanations provided by individuals for engaging in NSSI, such as overcoming negative emotions, achieving tension relief, or seeking social support, cannot readily be studied in non-human primates because of an inability to communicate their inner states.

Despite the fact that individuals with NSSI can verbalize the subjective consequences of performing acts of self-harm, we still know relatively little about the biological mechanisms that give rise to this disorder. Thus, a major strength of the present monkey model lies in its ability to fill this gap by providing detailed information on the neurobiological and genetic mechanisms underlying SIB, identifying the physiological and behavioral correlates of SIB, and developing novel treatment strategies. These findings have suggested new avenues for investigation in individuals with NSSI, some of which are now being implemented.

The value of any animal model depends on whether it is demonstrably valid with respect to the human condition. Nestler and Hyman (2010) discussed the three major kinds of validity of animal models in biological psychiatry. In face validity, the animal model should mimic the phenotype of the disorder in humans. In this regard, similarities are seen in both the forms and outcomes of self-harm in NSSI and SIB. Furthermore, in both monkeys and humans, the disorder typically arises spontaneously in adolescence, although it can also be triggered in response to stressful events in adults of both species. Construct validity refers to the concordances of biological dysfunctions associated with the etiology of the disorder and its neurobehavioral features. As discussed in this review, NSSI in humans and SIB in rhesus monkeys are commonly associated with early life stress or trauma, and individuals suffering from the respective disorders also show HPA axis dysregulation, abnormal social communication, and sleep disruption. Lastly, predictive validity refers to whether a model can predict the ability of treatments to induce remission. In the present case, this applies to pharmacotherapy, and findings indicate that naltrexone is one of the most effective drug treatments for both NSSI and SIB.

No animal is a perfect substitute for human beings. Despite the close genetic relationship between rhesus monkeys and humans, the two species differ in important ways. Compared to humans, the rhesus monkey cerebral cortex is not as well developed, their higher order cognitive capabilities are much more limited, and they have a different social structure. In addition to these general considerations, there are also specific limitations of the present animal model. One such limitation is its inapplicability to many populations that engage in NSSI. These include individuals with pervasive developmental disabilities or genetic disorders such as Lesch-Nyhan syndrome. Moreover, application of the rhesus monkey model to cases of NSSI comorbid with mood or anxiety disorders is probably incomplete, since the animal model does not attempt to recapitulate symptoms of depressive- or anxiety-like behaviors. These considerations highlight the heterogeneity of NSSI and the virtual impossibility of any single animal model to successfully reproduce all instances of this disorder. We also note that the predictive validity of the model with respect to pharmacotherapy is based almost entirely on drugs used to treat patients with borderline personality disorder. One of the major populations that we have attempted to model, specifically adolescents who engage in self-harm but have not been formerly diagnosed with any other psychiatric disorder, are not often referred for pharmacotherapeutic interventions.

In conclusion, this review has presented an animal model of NSSI that recapitulates many of the features of this behavioral disorder and that reasonably well satisfies the three main kinds of validity used in biological psychiatry. The need for a valid animal model of NSSI is prompted by two important facts. First, statistics show that this disorder is increasing in prevalence among the general population. Second, NSSI is often accompanied by suicidal ideation and, ultimately, by suicide attempts. Consequently, there is an urgent need for the medical community to learn how best to treat individuals with severe NSSI and, preferably, to prevent it from occurring in the first place.

AUTHOR CONTRIBUTIONS

MN and JM contributed equally to the conception of the idea, performing the bibliographic search, and drafting the article. Both authors approved the submitted version.

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Deliberative Decision-Making in Macaques Removes Reward-Driven Response Vigor

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Most of our daily decisions are governed by one of two systems: an impulsive system driving instantaneous decisions and a deliberative system driving thoughtful ones. The impulsive system reacts to immediately available concrete rewards. In contrast, the deliberative system reacts to more delayed rewards and/or punishments, which imposes consideration of longer-term choice consequences. Contingency management for addiction treatment is hypothesized to engage deliberative processes. Ultimately, in both decision-making situations, an action is needed to enact the decision. Whether those actions differ in implementation is an open question whose answer could inform as to whether distinct neural systems are engaged. To explore whether there is evidence of separate mechanisms between deliberated and immediate choices, we trained monkeys to perform a decision-making task where they made a choice on a touch screen between two visual cues predicting different amounts of reward. In immediate choice (IC) trials, the cues appeared at the final response locations where subjects could immediately touch the chosen cue. In deliberated choice (DC) trials, compound cues appeared orthogonally to the response locations. After a delay, allowing for decision formation, an identifying cue component was displaced to the randomly assigned response locations, permitting subjects to reach for the chosen cue. Both trial types showed an effect of cue value on cue selection time. However, only IC trials showed an effect of the competing cue on response vigor (measured by movement duration) and a reach trajectory that deviated in the direction of the competing cue, suggesting a decision reexamination process. Reward modulation of response vigor implicates dopaminergic mechanisms. In DC trials, reach trajectories revealed a commitment to the chosen choice target, and reach vigor was not modulated by the value of the competing cue. Our results suggest that choice–action dynamics are shaped by competing offers only during instantaneous, impulsive choice. After a deliberated decision, choice–action dynamics are unaffected by the alternative offer cue, demonstrating a commitment to the choice. The potential relevance to contingency management is discussed.

Keywords: decision-making, choice, deliberation, movement vigor, addiction

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INTRODUCTION

Recent decision-making theories posit that dynamics of enacting a decision are part of the decision-making process itself. Support for this comes from studies showing that, with immediate decisions, sensory evidence is accumulated across time prior to triggering an initial choice action. After action onset, subsequent sensory evidence can be used to confirm or possibly overrule the initial choice (Resulaj et al., 2009). These findings support a two-stage model of decision-making where post-decisional evidence can change the subject's confidence in the selected option, possibly leading to a change of mind (Pleskac and Busemeyer, 2010; Murphy et al., 2015). In contrast to immediate decisions, others might be deliberated before enactment. Contingency management, a successful approach for addiction treatment (Higgins et al., 2004), is hypothesized to engage deliberative decision-making (Regier and Redish, 2015). In this paper, we asked the empirical question of whether a two-stage model of decision enactment applies both when consequences of choice between two options of differing reward value are available immediately and also when the animal is forced to deliberate its choice. To do so, we conducted comparisons between action dynamics of immediate and deliberated decisions that might implicate differential engagement of neural systems relevant to the efficacy of contingency management.

MATERIALS AND METHODS

Subjects

Two adult male rhesus monkeys (*Macaca mulatta*), M1 and M2, were used in this study. Monkeys were housed in a temperature- and humidity-controlled vivarium that followed a 12-h light/dark cycle (light on at 7 a.m.). Animals were water regulated (30 ml/kg/day) in their home cage. Both animals contributed with 12 testing sessions (mean of 308 and 274 trials per sessions for M1 and M2). M1 was head fixed, allowing eye movement recordings. M1 contributed with 8 additional sessions (mean of 339 trials per session) in which hand movements were recorded (eye movements were not recorded in these behavioral sessions). All studies were approved by the NIDA-IRP Animal Care and Use Committee.

Training Procedure

Monkeys were trained to perform a decision-making task where they chose between two visual cues predicting different amounts of juice rewards. Importantly, they expressed their choice by touching the chosen cue on a touch screen. The use of a non-ballistic choice action is crucial as it can reveal a decision reexamination process as the response unfolds (Song and Nakayama, 2009). In immediate choice (IC) trials (**Figure 1A**, bottom timeline), monkeys released the hold button and reached for the chosen reward cue as soon as it appeared. In deliberated choice (DC) trials (**Figure 1A**, top timeline), subjects had to hold the button for a 2,200 ms deliberation period while reward cues could be compared, and the associated response symbol could be remembered. After a 1,000-ms delay during which only response symbols were visible, these symbols were repositioned, signaling the monkeys to release the button and touch the symbol associated with their chosen reward. During the testing sessions, IC and DC trials were randomly interleaved.

Prior to collecting the data reported in this paper, monkeys were successively trained to perform three tasks. A forced choice task, to learn the reward cue values, followed by a choice task to check the subject's understanding of the cue values, and finally a symbol association task. The trial structure of the forced choice task was very similar to IC trials. Briefly, in every trial, one reward cue was shown at one of the two possible response locations. The monkeys were allowed to release the hold button and reach for the reward cue as soon as it appeared in order to receive the corresponding amount of juice reward. After a few sessions, subjects were exposed to the choice task where the trial structure was identical to that in IC trials. Once the monkey's choice probability of choosing a high reward cue reached 0.8, the monkeys were trained on the symbol association task. The trial structure of the symbol association task was very similar to the one in DC trials. The only difference being that only one reward cue was shown and associated with one response symbol. Briefly, during cue onset, only one reward cue was shown, after which two symbols were randomly assigned and added, one surrounding the reward cue (e.g., diamond) and the other symbol (e.g., circle) shown at the opposite side of the monitor (no other reward cue was shown at that location). After the delay period (initially set to 50 ms and subsequently increased to 1,000 ms), the symbols were repositioned to the response locations, and the monkeys were allowed to reach for one of the two symbols. If the monkeys chose to reach for the symbol previously associated with the reward cue (diamond in this hypothetical case), they received the juice reward corresponding to the reward cue associated with it. If they chose to reach for the symbol not associated with the reward cue (circle in this hypothetical case), they were not rewarded. Once the monkey's choice probability of choosing the symbol associated with the reward cue reached 0.8, the monkeys were then tested on the final task (**Figure 1A**).

Behavioral Measurements

Behavioral control was implemented in the NIMH MonkeyLogic (Hwang et al., 2019). Choice responses were collected using a touch screen (Elo TouchSystems; Menlo Park, CA).

In every trial, subjects were given a choice between two reward cues signaling different amounts of juice reward units (**Figure 1B**, upper row) ranging from 0 (0 ml juice) to 4 (0.6 ml juice) units of reward. Reward distance (RD) is defined as the difference between units of reward signaled by the cues (see **Figure 1B**, lower row for three examples). In this paper, we only consider correct trials (trials where the high reward cue was chosen). All the data reported here are means across testing sessions, and the error bars show the standard errors of the means.

Figure 1C presents choice performance from trials across all Chosen Values, broken down by RD. In **Figures 2–4**, only trials where subjects chose the high reward cue signaling 2, 3, or 4 reward units (Chosen Value 2, 3, or 4) were considered, as only these trials offer at least 2 data points to measure the effect of RD

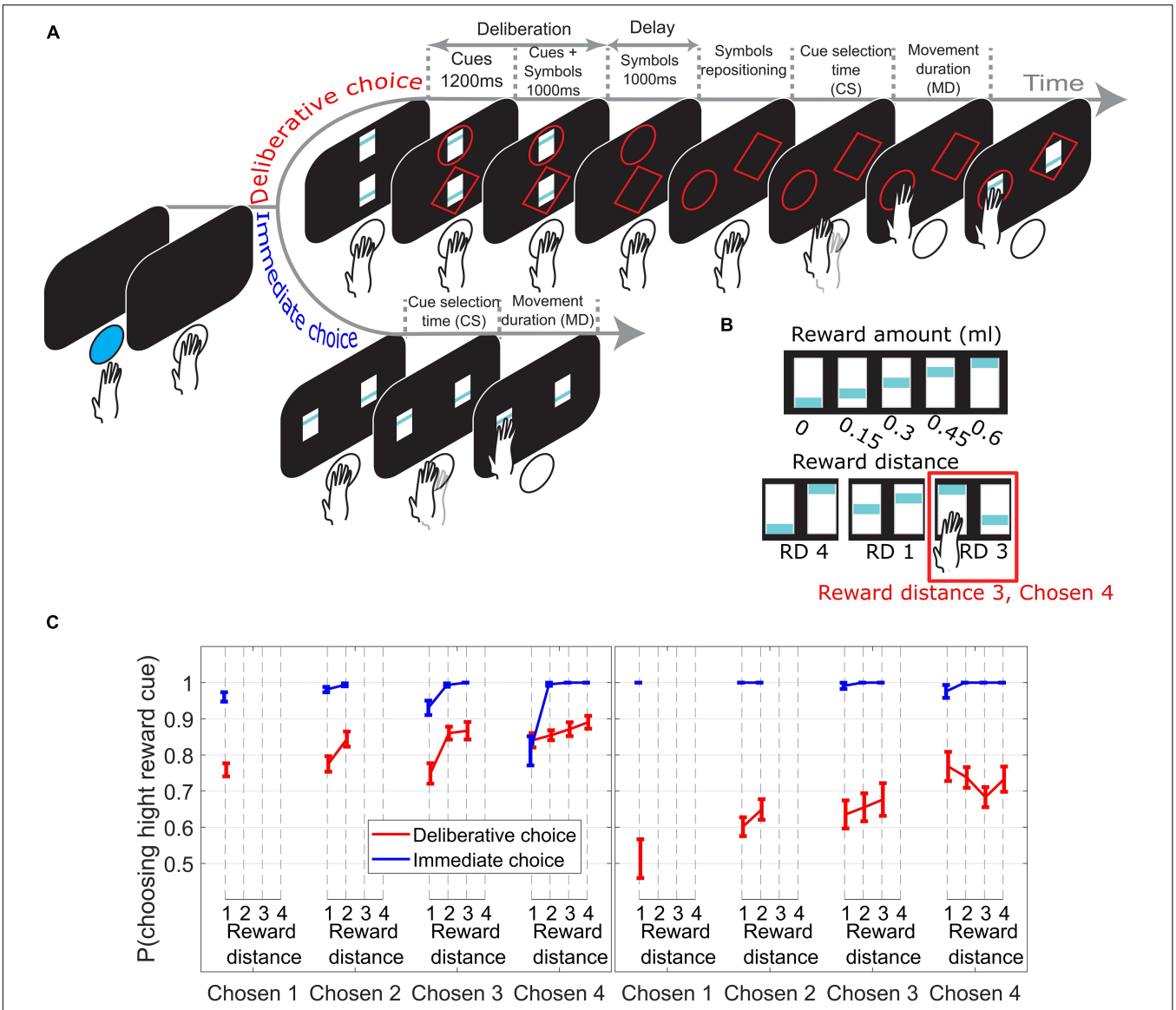

FIGURE 1 | (A) Subjects initiated a trial by pushing a blue led button. In DC trials (top timeline) two reward cues appeared, with the offer magnitudes 0–4 units of juice reward signaled by the blue bar height. Next, a circle or a diamond (response-symbol) was randomly assigned and added to each reward cue. Reward cues were removed during the delay period. Next, the response-symbols where repositioned randomly to the response locations allowing the subjects to reach for the symbol corresponding to their reward choice. The reward cue corresponding to the chosen symbol was shown for an additional 300 ms as visual feedback and reward was delivered. In IC trials (bottom timeline), subjects reached for the chosen reward cue as soon as it appeared. **(B)** Upper row shows the reward cues used with their corresponding amount of juice reward. Lower row shows 3 example trials with the corresponding reward distance (RD) between cues. **(C)** Choice performance across sessions (± SEM) as function of reward distance between the choice offer cues for different Chosen Values. Left/right panel from M1/M2.

on the measured behavioral output (i.e., cue selection time and movement duration).

We measured 3D hand movements using an inertial measurement unit (NGIMU, x-io Technologies, United Kingdom) sampling at 240 Hz (Madgwick et al., 2011) attached to the monkey's right wrist. In brief, gyroscope and accelerometer data were combined to compute rotational and translational wrist movement acceleration in a gravity-centered frame of reference. The 3D acceleration data were double integrated to get the 3D hand trajectories (Madgwick et al., 2011). Next, 3D hand reach trajectories to the left and right targets were projected onto a plane fitted to the raw data. With the resulting 2D data, we computed the maximum reach deviation distance between a straight line connecting the start and the end position and the actual hand trajectory for each trial type and RD. We defined the deviation as negative/positive when the trajectory was curved away/toward the unchosen cue.

We monitored eye position using an infrared system (ISCAN ETL-200) sampling at 240 Hz. For the analysis, the recorded horizontal and vertical eye position traces were smoothed

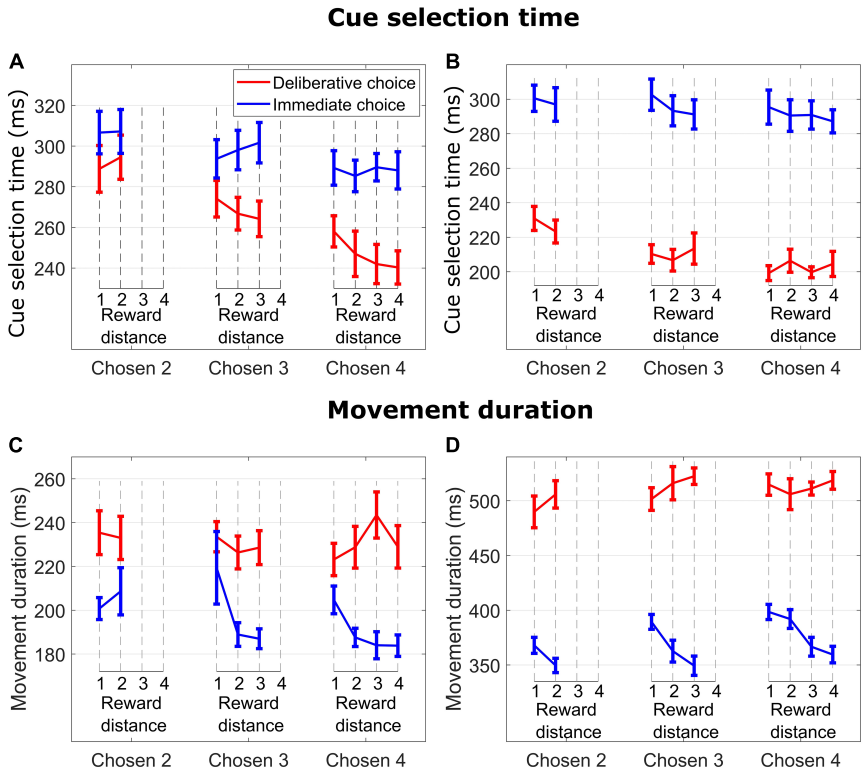

FIGURE 2 | (A) Cue selection time (time from when the cues were presented to when the monkey released the hold button) vs. reward distance (RD) between cues for different Chosen Values for DC (red curves) and IC (blue curves) trials for M1. **(B)** Same format as in **(A)** for M2. **(C)** Movement duration (time from when the monkey released the hold button to when the monkey touched the reward cue) vs. reward distance (RD) between cues for different Chosen Value for DC (red curves) and IC (blue curves) trials for M1. **(D)** Same format as in **(C)** for M2.

with a Savitzky–Golay filter (window = 10 data points, polynomial degree = 4). The resulting filtered eye traces were subjected to a non-parametric algorithm using k-means cluster analysis to extract saccade reaction times and fixation locations (König and Buffalo, 2014).

RESULTS

Reward Choice

On IC trials (**Figure 1C**, blue lines), subjects chose the high reward cue with the same high probability irrespective of the Chosen Value (linear regression of p(choose high cue) on Chosen Value collapsed across all RDs; M1: $p = 0.17$; M2: $p = 0.24$). In DC trials, however (**Figure 1C**, red lines), the probability of choosing the symbol associated with the high reward cue increased when the value increased (linear regression of p(choose high cue) on Chosen Value, collapsed across all RDs; M1: $p = 1.3e{-}05$; M2: $p = 3.74e{-}07$). The effect of RD on the probability of choosing the high reward cue was not consistent between the two monkeys on DC trials.

Cue Selection Time

On IC trials, subjects quickly made a decision and selected the reward cue corresponding to their initial choice. At a given Chosen Value (2, 3, or 4), cue selection time (time from when the cues were presented to when the monkey released the hold button) was not modulated by RD (blue lines in **Figure 2A** for M1, all $p > 0.55$; blue lines in **Figure 2B** for M2, all $p > 0.35$), suggesting that choice initiation did not involve an ongoing consideration of the unchosen cue. On the other hand, cue selection time slightly decreased as Chosen Value increased. This effect was significant with subject M1 but did not reach significance level with subject M2 (linear regression of CS time on Chosen Value, collapsed across RDs; M1: $p = 0.014$; M2: $p = 0.27$), suggesting that choice action initiation was not consistently influenced by Chosen Value.

In DC trials, subjects had presumably enough time to make a decision during the 2,200-ms deliberation period. When the response symbols were randomly repositioned to the response locations, subjects searched and selected the response symbol (circle or diamond) corresponding to their chosen reward cue. As in IC trials, DC trials featured an absence of RD influence on cue selection time (red lines in **Figure 2A** for M1, all $p > 0.15$; red lines in **Figure 2B** for M2, all $p > 0.43$), suggesting again an absence of ongoing consideration of the unchosen cue when initiating the choice action. Cue selection time decreased as Chosen Value increased (linear regression of cue selection time on Chosen Value collapsed across all RDs; M1: $p = 1.46e{-}07$; M2: $p = 2.49e{-}05$).

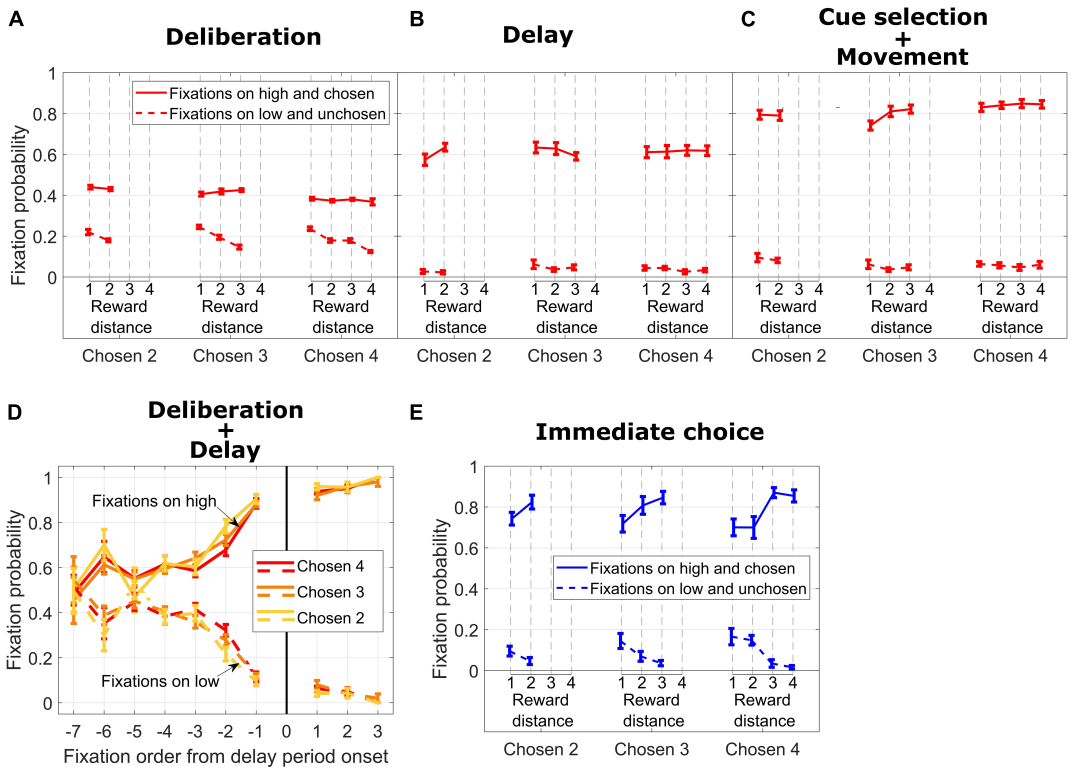

FIGURE 3 | (A) Fixation probability on high/chosen (solid lines) and low/unchosen (dotted lines) cues vs. reward distance (RD) for different Chosen Values during DC trials, during the deliberation period. **(B)** Same format as in **(A)** for the delay period. **(C)** Same format as in B for cue selection and movement period. **(D)** Fixation probability on high/chosen (solid lines) and low/unchosen (dotted lines) cues for different Chosen Values vs. fixation order from end of the deliberation period. **(E)** Same format as in **(C)** for IC trials.

The influence of Chosen Value on cue selection time was greater in DC then in IC trials. This effect was significant with subject M1 and was close to be significant with subject M2 (i.e., the cue selection time difference between Chosen Values 3 and 4 was greater in DC than in IC trials; linear regression of cue selection time on Chosen Value, with TrialType × Chosen Value interaction term, M1: p = 0.02; M2: p = 0.08). This suggests that cue selection was more sensitive to Chosen Value when the decision was deliberated, and expected reward value was known.

We also compared cue selection time on correct and error trials (**Supplementary Figures 1A,B,E**). Only trials where subjects chose a symbol associated with cues signaling 1, 2, or 3 reward units were included (trials highlighted with gray background in **Supplementary Figure 1A**), as only those trials offer data points from both trial types: Chosen Value 0 only offers data from error trials, whereas Chosen Value 4 only offers data from correct trials. For a given Chosen Value, the data were collapsed across all RDs. For both subjects (**Supplementary Figure 1A** for M1 and **Supplementary Figure 1B** for M2; **Supplementary Figure 1E** for M1 during the hand tracking sessions), cue selection time was shorter on error trials when subjects chose a symbol associated with cue signaling 1 unit of reward in most of the testing sessions (Wilcoxon rank sum test comparing medians of cue selection time on correct and error trials for Chosen Value 1; M1, p = 0.012; M2, p = 0.004; M1 hand tracking sessions, p = 0.1). Importantly, cue selection time was sensitive to Chosen Value only when subjects made a correct choice (linear regression of cue selection time on Chosen Value, collapsed across all RDs; M1: correct trials p = 0.0002, error trials p = 0.36; M2: correct trials p = 3.8e−05, error trials p = 0.36; M1 hand tracking sessions: correct trials p = 0.002, error trials p = 0.18). These findings suggest that choice errors made by subjects are due to a failure to deliberate or a failure to remember the symbol associated with the high reward cue.

Taken together, in both decision-making scenarios, cue selection time on a manual choice task seems to be insensitive to the value of the unchosen cue, suggesting that this period of time did not involve an ongoing cue comparison process. On the other hand, cue selection time was sensitive to the absolute value of the chosen cue. Taken together, the data so far suggest that choice initiation is compatible with literature showing reaction time sensitivity to the value of the reach target (Opris et al., 2011; Mosberger et al., 2016; Summerside et al., 2018).

Movement Duration

There was an effect of RD on movement duration on IC trials when subjects chose and reached for cues signaling 3 and 4 reward units (blue lines in **Figure 2C** for M1, Chosen

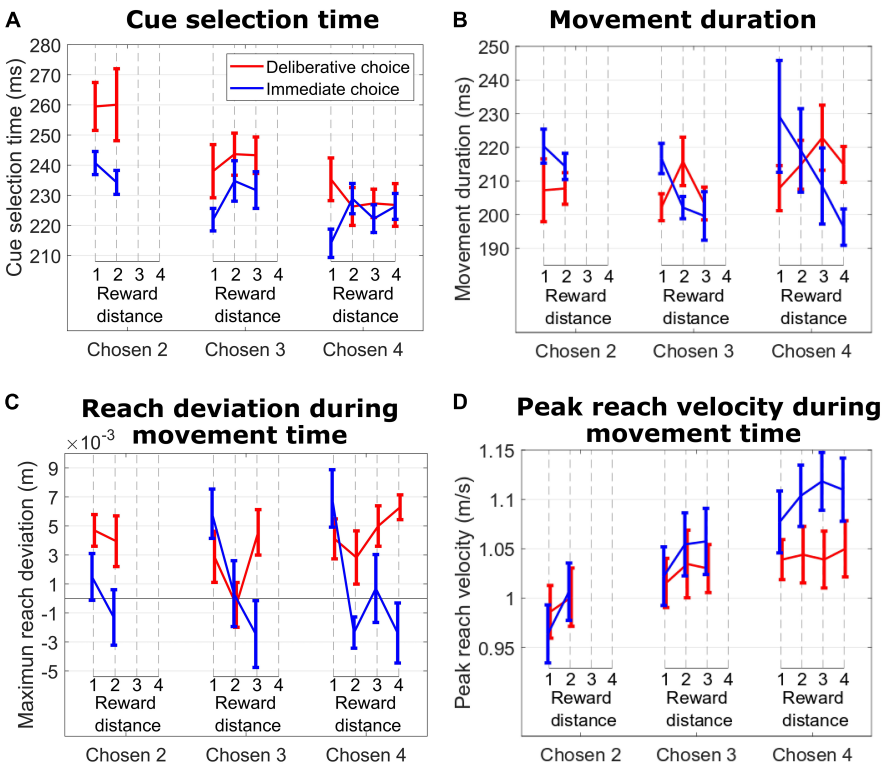

FIGURE 4 | (A) Cue selection time vs. reward distance (RD) between cues for different Chosen Values for DC (red curves) and IC (blue curves) trials during the hand tracking sessions with subject M1. **(B)** Same format as in **(A)** for movement duration. **(C)** Same format as in B for maximum reach deviation. **(D)** Same format as in **(C)** for peak reach velocity.

Value 4: $p = 0.009$; Chosen Value 3: $p = 0.035$; Chosen Value 2: $p = 0.51$; blue lines in **Figure 2D** for M2, Chosen Value 4: $p = 0.0002$; Chosen Value 3: $p = 0.002$; Chosen Value 2: $p = 0.07$). Specifically, subjects took more time to reach and touch a reward cue when the RD was low (hard decision), suggesting consideration of the unchosen cue throughout the reach movement period (see below data from hand movement tracking sessions).

In contrast, DC trials did not exhibit any influence of RD on movement duration when reaching for cues signaling 2, 3, or 4 reward units (red lines in **Figure 2C** for M1, all $p > 0.44$; red lines in **Figure 2D** for M2, all p-values > 0.2), nor did the value of the chosen cue (linear regression of movement duration on Chosen Value, collapsed across all RDs; M1 $p = 0.75$; M2 $p = 0.17$). These findings suggest an absence of consideration of the unchosen cue throughout the reach movement period when the choice was deliberated, indicating a commitment to the initial choice decision.

DC trials did not exhibit any influence of Chosen Value on movement duration on either correct or error trials (**Supplementary Figures 1C,D** for M1 and M2, respectively, **Supplementary Figure 1F** for M1 during the hand tracking sessions; linear regression of cue selection time on Chosen Value, collapsed across all RDs (M1: correct trials $p = 0.47$, error trials $p = 0.52$; M2: correct trials $p = 0.09$, error trials $p = 0.36$; M1 hand tracking sessions: correct trials $p = 0.97$, error trials $p = 0.48$). Given a Chosen Value of 1, 2, or 3, movement duration did not differ between correct and error trials (Wilcoxon rank sum test comparing medians of movement duration for Chosen Value 1, 2, or 3; M1, all $p > 0.05$; M2, all $p > 0.05$; M1 hand tracking sessions, all $p > 0.05$ except Chosen Value 2, $p = 0.007$).

Taken together, even though subjects made a choice to reach for a cue/symbol signaling the same amount of reward in both trial types, the cue selection process and the action implementing the choice differ in several respects. We show that, when subjects made an immediate decision (IC trials), cue selection time was insensitive to the unchosen cue value, but after the choice initiation, the value of the unchosen cue influenced movement duration, suggesting a choice revision process emerges in which the value of the competing cue either confirms or interferes with the initial decision. On the other hand, when subjects deliberated on the choice (DC trials), cue selection time was more sensitive to the target's absolute value, but insensitive to the unchosen cue value.

The Decision-Making Process

To what extent can we be sure that subjects came to a decision during the deliberation period on DC trials? Potentially, subjects could simply remember the values associated with each symbol (circle or diamond) and make their choice only after the go signal was given. To answer this, we analyzed eye movement data from subject M1. **Figure 3A** shows fixation probability on high

(chosen) vs. low (unchosen) reward cues during the deliberation period (in **Figure 1A**, upper timeline, the deliberation period encompasses the "Cues" and "Cues + Symbols" periods of time). In general, fixation probability on the high and ultimately chosen reward cue was higher than fixation probability on the low and unchosen cue. When we sorted the trials according to Chosen Value, we did not find any significant effect of RD on fixation probability on high and chosen cues (solid red lines in **Figure 3A**, all $p > 0.18$). In contrast, fixation probability on low and unchosen cues decreased with increasing RD (dotted red lines in **Figure 3A**, Chosen Value 4: $p = 2.04e{-}10$; Chosen Value 3: $p = 2.37e{-}08$; Chosen Value 2: $p = 0.01$).

During the subsequent delay period, fixation probability on symbols corresponding to the high and chosen cues increased dramatically for all values of the chosen symbol and did not depend on RD (**Figure 3B**, solid lines, all $p > 0.09$). Fixation probability on the unchosen cue decreased nearly to zero, and the dependency on RD disappeared (**Figure 3B**, dotted lines, all $p > 0.26$). This pattern of fixations, in our opinion, signals a termination of the decision-making process. During this time frame, the subject also occasionally fixated on the response locations where the symbols were to be repositioned (data not shown).

Probability of fixation on the chosen cue increased even more during the subsequent time period encompassing cue selection and movement duration time periods and mostly were independent from RD (**Figure 3C**, solid lines, Chosen Value 4: $p = 0.54$; Chosen Value 3: $p = 0.02$; Chosen Value 2: $p = 0.9$). Also, fixation probability increased with Chosen Value (linear regression of fixation probability on Chosen Value, collapsed across all RDs; $p = 0.004$), showing a facilitation of selecting the high reward cue by the eye. Additionally, fixation probability on the unchosen cue stayed low and did not depend on RD (**Figure 3C**, dotted lines, all $p > 0.5$) nor on the absolute value of the chosen cue (linear regression of fixation rate on Chosen Value, collapsed across all RDs, $p = 0.06$).

To investigate the temporal aspect of fixation allocation during the deliberation period and the subsequent delay period, we calculated the probability of fixation on the chosen and unchosen reward cues (**Figure 3D**). As shown in **Figure 3C**, irrespective of Chosen Value, the subject fixated both cues with the same probability at the beginning of the deliberation period (from Fixations −7 to −5 in the x-axis in **Figure 3D**; Wilcoxon rank sum test comparing medians of fixation probability on high and low reward cues for a given Chosen Value and fixation order: Chosen Value 4, Fixation −7, $p = 0.87$; Fixation −6, $p = 0.02$; Fixation −5, $p = 0.11$; Chosen Value 3, Fixation −7, $p = 0.63$; Fixation −6, $p = 0.03$; Fixation −5, $p = 0.17$; Chosen Value 2, Fixation −7, $p = 0.8$; Fixation −6, $p = 0.02$; Fixation −5, $p = 0.98$). When considering the last four fixations during the deliberation period (Fixations −4 to −1), we found that the subject fixated the ultimately chosen reward cue more from Fixations −4 to −1 combined (Wilcoxon rank sum test comparing medians of fixation probability on high and low rewards: Chosen Value 4, $p = 1.4e{-}08$; Chosen Value 3, $p = 3.09e{-}09$; Chosen Value 2, $p = 1.6e{-}08$). This fixation bias increased sharply from Fixations −4 to −1 to reach a probability of fixating the high reward cue of 0.88 (Chosen Values 3 and 4) and 0.9 (Chosen Value 2). The first fixations on symbols associated with cues at the beginning of the delay period (Fixation 1 in the x-axis in **Figure 3D**) were predominantly on the symbol associated with the ultimately chosen high reward cue. This bias did not progress (from Fixations 1 to 3 in the x-axis in **Figure 3D**) as sharply as during the deliberation period, suggesting a commitment to choose the symbol associated with the high reward cue.

For IC trials, we considered all fixations on cues during a time window encompassing the cue selection and movement duration times (see **Figure 1A** lower timeline). As shown in **Figure 3E**, fixation probability on the high and ultimately chosen reward cue was higher than fixation probability on the low reward cue across all Chosen Values. Additionally, fixation probability on the ultimately chosen cue increased with RD for most of the Chosen Values (solid blue lines in **Figure 3E**, Chosen Value 4: $p = 0.0005$; Chosen Value 3: $p = 0.0003$; Chosen Value 2: $p = 0.42$). Fixation probability on the low unchosen reward cue decreased as RD increased for the higher Chosen Values (dotted blue lines in **Figure 3E**, Chosen Value 4: $p = 0.0001$; Chosen Value 3: $p = 0.02$; Chosen Value 2: $p = 0.28$). Taken together, when the subject faced an easy decision (high RD), fixation probability on the high reward cue was the highest and fixation probability on the low reward cue was the lowest. On the other hand, when the subject faced a hard decision (low RD), those two fixation probabilities were closer to each other, suggesting greater decision uncertainty requiring visual comparison.

Choice Action Dynamics

To shed light on how the initial decision might be revised during choice action, we recorded 3D hand movements from subject M1 in separate sessions while performing the exact same task as in **Figure 1A**. The effects of RD and Chosen Value on cue selection time and movement duration largely replicated the effects seen in both monkeys without the wrist accelerometers and are summarized in **Table 1**.

The effect of RD on movement duration in IC trials suggested an emerging consideration of the unchosen cue as a possible choice target during the reach movement. This decision revision was absent when we analyzed the effect of RD on movement duration in DC trials, suggesting a commitment to the choice. Support for this interpretation comes from hand trajectory deviations and peak reach velocity. Specifically, during IC trials (**Figure 4C**, blue lines), we show that the subject's hand trajectory deviated in the direction of the competing cue, and the amplitude of that deviation was greatest on trials with the smallest RD (hard trials). This effect was more pronounced when the subject reached for cues signaling 3 and 4 units of reward (Chosen Value 4: $p = 0.017$; Chosen Value 3: $p = 0.014$; Chosen Value 2: $p = 0.31$). When we considered DC trials (**Figure 4C**, red lines), the hand deviation was consistently positive (probably reflecting an idiosyncratic behavior), but importantly, this deviation did not depend on RD when the subject reached for cues signaling 2, 3, or 4 units of reward, suggesting an absence of consideration of the unchosen cue during the reach movement (all $p > 0.19$). The absolute value

TABLE 1 | Upper table: *p*-values of linear regression of RD on CS time and MD for IC and DC trials (first rows) during the 3D hand tracking session.

		Cue selection time			Movement duration		
		Chosen4	**Chosen3**	**Chosen2**	**Chosen4**	**Chosen3**	**Chosen2**
Immediate choice trials (IC)	RD effect	0.17	0.23	0.26	0.04	0.03	0.36
	Chosen value effect		0.001			Regression not performed	
Deliberative choice trials (DC)	RD effect	0.38	0.6	0.9	0.37	0.89	0.96
	Chosen Value effect		7.9e–06			0.13	
		Cue selection time vs. Chosen Value					
Trial type (IC vs. DC)		0.03					

p-values of RD chosen cue value on CS time and MD for IC and DC trials (2nd rows). Lower table: Linear regression of CS time on Chosen cue value, with TrialType X ChosenValue interaction term during the 3D hand tracking session, p = 0.03.

of the chosen target had a significant effect on peak reach velocity on IC trials, suggesting a motivational effect on choice action vigor (linear regression of peak velocity on Chosen Value, collapsed across all RDs; $p = 0.01$). This effect was absent on DC trials ($p = 0.23$).

DISCUSSION

We have shown that cue selection and choice action dynamics differ when the decision maker is forced to deliberate a decision prior to responding (DC), vs. enacting the choice response during decision formation (IC).

In IC trials, the unleashing of the initial decision did not involve an ongoing cue comparison process, as shown by the lack of influence of RD on cue selection time. However, after initiating the choice decision, IC trials revealed a continuing refinement of the initial choice throughout the post-decisional/action epoch. Specifically, reach duration was longer when subjects were confronted with a hard decision (low RD), suggesting a more pronounced competition between reward cues for selection. This competition was also reflected in the hand trajectory as it deviated more in the direction of the competing cue when the competition was harder to resolve. On top of the influence of the competing cue on action dynamics, the Chosen Value regulated the movement vigor (indexed here by peak reach velocity).

In DC trials, subjects made an initial, committed choice. As in IC trials, the unleashing of the decision did not involve an ongoing cue comparison process. After initiating the choice and in contrast to IC trials, DC trials revealed a continued absence of cue competition, as it took the subjects the same amount of time to reach and touch the symbol corresponding to the chosen reward irrespective of RD. This commitment to reach and touch the chosen symbol was also reflected by the absence of deviation in hand trajectory toward the competing symbol when subjects faced hard (low RD) or easy (high RD) decisions. Moreover, response vigor was not modulated by the expected value of the choice action.

In DC trials, the eye movement data during the deliberation and the subsequent delay periods revealed that the animal did indeed consider both choice options at the beginning of the deliberation. After a few fixations, the subject discarded the low reward cue as a possible choice. When the go signal was given, the eye and the hand selected the high reward symbol, and this cue selection for both effectors was modulated by the absolute value of the symbol corresponding to the chosen cue, but not by the value of the competing cue. Cue selection time was reduced on trials with greater Chosen Value (**Figures 2A,B**), while the eye selected the high cue with higher probability (**Figure 3C**). On the other hand, in IC trials, when the reward cues appeared, both effectors showed an effect of the value of the unchosen cue. More precisely, both fixation probability (**Figure 3E**) and hand movement duration (**Figures 2C,D**) depended on RD. These data are compatible with literature (Khan et al., 2011; Nissens and Fiehler, 2018) showing a shared single attentional resource between the hand and the eye when planning a visually guided reach movement. Thus, on IC trials, the attentional drive continued to extract information pertinent to the choice in a way that gave the unchosen cue influence over action dynamics. In DC trials, attention deployed to select the high reward cue without influence of the unchosen cue.

In both trial types, cue selection time depended on the absolute value of the chosen cue. On IC trials, movement duration depended on RD and Chosen Value, whereas on DC trials, it did not depend on either. This dissociation of the influence of the decision-making context on cue selection time and movement duration has been reported with human subjects (Wong et al., 2015), suggesting that cue selection time and movement duration are independent movement parameters.

The observed difference in choice action vigor regulation for the two decision-making situations is indicative of the involvement of separate decisional systems. More precisely, it has been shown that decisions involving an immediately available reward are associated with activation of the midbrain dopamine system (McClure et al., 2004), which has also been shown to causally regulate action vigor (Niv et al., 2007; Beierholm et al., 2013; da Silva et al., 2018; Mendonça et al., 2021). Thus, we believe that our IC trials unleash an impulsive decisional system (Dayan et al., 2006) that incorporates neural systems purported to support the ability of drug cues to trigger craving and relapse (Phillips et al., 2003). On the other hand, the lateral prefrontal cortex has been shown to support decisions yielding a more distal and abstract reward (McClure et al., 2004). Therein, the predicted

value of the chosen option (which was reflected in cue selection time in DC trials) was causally encoded (Lak et al., 2020). This brain region has also been shown to support imagination and evaluation of future outcomes, two important attributes of deliberation (Schacter et al., 2017).

In addiction, an impulsive decisional system, including dopaminergic elements, is believed to drive behavior (Volkow et al., 2008, 2017). Contingency management, an effective treatment for drug addiction (Higgins et al., 2004), is thought to temper this behavioral drive by providing an alternative reinforcer (money) that forces subjects into a deliberative mode (Regier and Redish, 2015). In light of our findings, we speculate that deliberation engaged by contingency management helps subjects to make decisions based on a considered evaluation of options: drug taking with negative consequences vs. money. Importantly, as we show here, those deliberated decisions appear to disengage dopaminergic systems known to modulate reward-driven vigor and that are believed to support drug craving.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the NIDA-IRP Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

ND, HJ, and CB designed the research and wrote the manuscript. ND performed the research. ND and HJ analyzed the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2021.674169/full#supplementary-material

Supplementary Figure 1 | (A) Cue selection time vs. Chosen Value in DC trials for correct (dark red curves) and error (light red curves) trials during the main data set sessions with subject M1. **(B)** Same format as in **(A)** for subject M2. **(C)** Same format as in **(A)** for movement duration for subject M1. **(D)** Same format as in **(C)** for subject M2. **(E)** Same format as in **(A)** during the hand tracking sessions with subject M1. **(F)** Same format as in C during the hand tracking sessions with subject M1. The trials highlighted with shaded area in A were used to compute the effect of the chosen cue value on cue selection time and movement duration.

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