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A novel model of drug cue-induced behaviours in rhesus macaque subjected to chronic ketamine exposure

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To the Editor,

Non-human primate (NHP) models are advantageous for mimicking human addiction with high behavioural validity.[1] However, current NHP drug addiction models (eg, self-administration) often require a comprehensive behavioural training paradigm, relatively expensive apparatus and invasive surgical procedures. These obstacles have significantly reduced the feasibility and application of NHP research on substance use disorders or drug addiction. In recent decades, ketamine has become a popular recreational drug among young adults. However, although very low doses of ketamine have been used as a rapid-acting antidepressant,[2 3] the serious harmful consequences of its non-medical use as a 'club drug' remain a public health concern. Here, we report that drug-associated cues efficiently evoke addiction-like behaviours in rhesus macaques receiving a chronic dosing paradigm of ketamine, and further refined the quantification procedure of the behavioural assessment standards. Furthermore, our findings provide a relatively simplified behavioural model for mimicking ketamine addiction and investigating novel therapeutic approaches (see figure 1A (1–3)).

As illustrated in figure 1A (1), we used three male rhesus macaques that had previously received two doses of ketamine monthly for 2 years (1 mg/kg intramuscularly), which meets the requirements for assessing the long-term use of moderate/low ketamine dosing. These animals subsequently received daily ketamine injections (1 mg/kg intramuscularly daily) for 1 month under single-housing conditions to advance the escalation pattern of increasing dosage and the frequency of drug intake. Single-housing conditions contribute to a moderate increase in stress levels and may facilitate the emergence of drug-associated behavioural changes.[4] They also enable individual video monitoring and follow-up evaluations of addiction-related behavioural parameters. As a control, we used a single male rhesus macaque that had not been exposed to ketamine.

Drug addiction is characterised by cue-induced drug use and relapse,[5] and consequently, we standardised the procedure of drug-cue presentation as a key step in our evaluation of addiction-like behavioural features. During this process, the experimenter enters the home cage and stands facing the animal at a distance of 1 m. The experimenter then reveals a syringe previously hidden behind his/her back. Interestingly, after 1 month of daily injections under single-housing conditions, all three animals rapidly exhibited drug-approaching behaviours as prominent behavioural responses to cue presentation, which entailed turning their rears towards the experimenter as a sign of 'wanting' behaviour.

To fully characterise the cue-evoked behavioural responses and validate the model for treatment evaluation, we further performed behavioural mapping based on continuous video recording using a high-definition digital camera (FDR-AX45 4K; Sony, Japan). In this regard, we developed an optimised scoring system for addiction-related behavioural severity. Video recordings were evaluated by three trained observers blinded to the treatment conditions, and following cue presentation (5 s), five types of behaviours were identified and scored as follows:

1. Approaching, indicating that the animal will present its rear for eliciting injection, or move its body towards the experimenter. This behaviour mimics cue-induced drug-seeking behaviour, such as lever pressing in the self-administration paradigm.

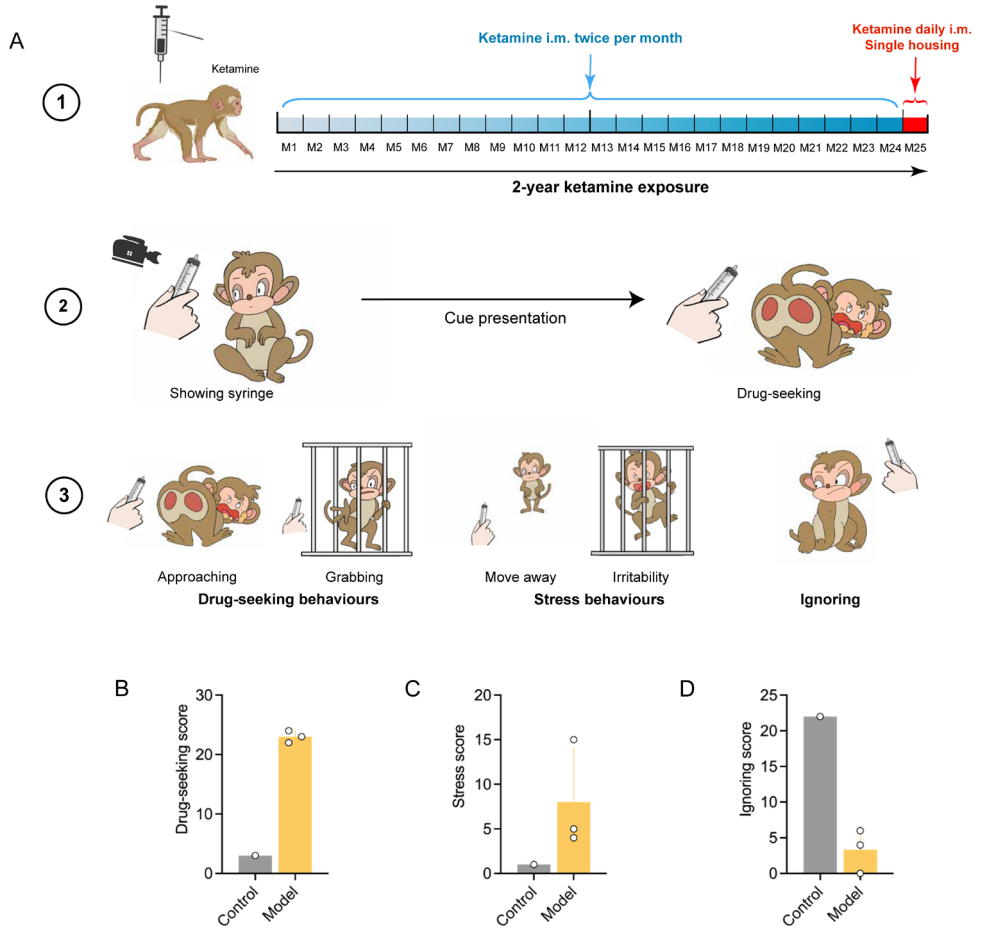

Figure 1 A schematic representation of a macaque model of chronic ketamine exposure and evaluation procedures, and behavioural scores after a discontinuation of ketamine exposure. (A(1)) A flowchart showing the establishment of a ketamine chronic dosing regimen. (A(2)) The drug-associated cue presentation procedure of behavioural tests. (A(3)) Representative cue-evoked behavioural responses, which are used for score evaluation. (B) Scores of drug-seeking behaviour. (C) Scores of stress responses. (D) Scores of ignoring behaviour. i.m., intramuscularly.

2. Grabbing, with limbs reaching out of the cage fence, indicating that the animal is excited. This behaviour may appear sequentially after approaching, which reflects the emotional status of 'wanting', such as the strong impulsivity of drug seeking.
3. Behavioural withdrawal, retreating to the rear of the cage, and standing with a tense posture, indicating evasion of the stimulus, which may reflect a stress response.
4. Irritability, manifested as cage shaking and/or the bearing of teeth, as a show of defensive behaviour.
5. Ignoring, such as inattention or disregarding the cue (syringe).

Score calculation: Each behavioural response that persisted for longer than 2 s was recorded as a single action and was assigned 1 point for every 2 s of its duration. A response interval lasting longer than 2 s was regarded as a cessation of the response. For each behavioural category, the total score was the sum of all behavioural scores during a single test session.

In this study, we thus established a chronic ketamine dosing regimen combined with environmental stress to trigger and evaluate the drug cue-associated addiction-like behaviours of rhesus macaques (see figure 1B–D). The ketamine-exposed macaques showed a significant increase in drug-seeking (approaching and grabbing) and stress (withdrawal and irritability) behaviours. On the other hand, the ignoring behaviours decreased, indicated an indifference towards the drug-paired cue. In this context, it has previously been demonstrated that monkeys subjected to 6 months rather than 1 month of consecutive intravenous ketamine injections were characterised by neuronal apoptosis in the prefrontal cortex and abnormal motor behaviours.[6] Moreover, a neuroimaging study revealed that the brains of monkeys receiving the same treatment displayed hyperactivity in the entorhinal cortex and striatum, together with hypoactivity in the visual cortex and midbrain, all of which are key regions associated with addiction and stress behaviours.[7] Those findings indicate that obvious pathological changes in monkeys tend to develop over a relatively long-term rather than in the short-term exposure to ketamine. Considering that the criterion used to define long-term ketamine users or abusers in most clinical studies is typically more than 2 years, we believe that periodic ketamine exposure over an extended period is preferable for NHP models designed to simulate the recreational use of ketamine in humans. Although a 2-yearparadigm is ostensibly long, a better-constructed

animal model with face validity will contribute considerably to enhancing research efficiency and also reduce the number of experimental animals needed. Thus, despite the diversity of drug usage patterns in humans, we have adopted a chronic dosing schedule for 2 years, followed by more frequent administration over a single month, thereby mimicking the loss of control in repeated ketamine use. This procedure and dosage are safe for NHPs and comparable to the reported clinical cases of ketamine abuse. Additionally, single-housing conditions have been established to elevate static stress levels that may promote the emergence of drug-seeking behaviours and also facilitate the monitoring of individual behaviours captured by continuous video recording. Our protocol could be practicably applied in most NHP facilities and could be combined with other home-cage analyses, including those used to assess gross motor activity, food intake, grooming, and to monitor associated changes (e.g., mood status and sleep rhythm) following chronic ketamine dosing. The development of an NHP chronic ketamine exposure model is timely for gaining a better understanding of ongoing ketamine abuse and assessing novel therapeutic interventions for ketamine addiction treatment approaches.

In summary, we have developed an NHP model and provided preliminary evidence for evaluating drug cue-associated behavioural responses to ketamine, the abuse of which continues to grow worldwide.[8] Our model, combined with neuroimaging, neurophysiological, and neuropathological assessments, may contribute to a better understanding of the chronic effects of ketamine, and it may also serve as a tool for assessing treatment interventions.

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